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# METABOLIC REGULATION AND MATHEMATICAL MODELS

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## I. INTRODUCTION

Since the identification of the fundamental pathways of intermediate metabolism some 30 years ago it has been recognized that the stage has been set for the study of the regulation of metabolism. A multitude of experimental investigations have yielded insight into various principles of regulation such as induction and repression of enzymes, modulation of their activity by covalent modification and by allosteric effectors, the distributory function of coenzymes and metabolites and so on. On the other hand there has grown a sizable literature on the theoretical analysis of biological regulation, mostly by means of mathematical models. There is no denying the fact that there exists a dichotomy, with the experimenters taking little cognizance of the results of the theoretical work while much of the modeling is far removed from concrete biological systems. It is the purpose of the present survey to help bridge this chasm—or, if it fails, to land between the two chairs—by trying to do justice to both the viewpoints of the modeler and the experimenter. It will try to keep biological questions always in the center while maintaining mathematical rigor. It is not intended to deal with a wide range of biological systems and levels. It will concentrate essentially on the energy metabolism of eukaryotes neglecting both the epigenetic level and the intercellular interactions. We shall not attempt to survey the entire variety of mathematical models proposed. The validity and utility of a given model should be judged in the long run by the experimenters in that field. Since, however, the methods of setting up models have much in common their general principles will be presented in such a way that they can be applied without undue difficulty by the non-expert in mathematics. The application of the methods will be shown on two kinds of examples, for one on simplified biochemical systems and secondly, on glycolysis, especially of the erythrocytes, as a concrete biological system. The first type serves mainly to clarify the mathematical aspects and to derive some biological principles, the latter to demonstrate some of the biological conclusions which may be drawn. The analysis of the dynamics of the systems includes the conditions under which oscillations may arise or be suppressed. Furthermore, the problems of control of metabolic systems and the methods to identify and assess the controlling steps are considered.

## II. MODELING AS A METHOD FOR THE STUDY OF THE METABOLIC REGULATION

### 1. *Some Characteristics of Biological Systems*

Since modeling aims at a deeper understanding of real systems reasonable models have to represent essential qualities of the objects they simulate. Given this starting

ABBREVIATIONS

*Metabolites*: G6P, glucose 6-phosphate; F6P, fructose 6-phosphate; $FP_2$, fructose 1,6-biphosphate; GAP, glyceraldehyde 3-phosphate; DHAP, dihydroxyacetone phosphate; triose-P, triose phosphate (sum of GAP and DHAP); $1,3P_2G$, 1,3-bisphosphoglycerate; 3PG, 3-phosphoglycerate; 2PG, 2-phosphoglycerate; PPyr, phosphoenolpyruvate; Pyr, pyruvate; Lac, lactate; 2,3-bisphosphoglycerate; $GP_2$, glucose 1,6-bisphosphate; Mg·ATP, 1:1 complex of $Mg^{2+}$ with ATP.

*Enzymes*: In the formulae and figures the following abbreviations of enzymes are used for identification: HK, hexokinase (EC 2.7.1.1); PGI, phosphoglucoisomerase (EC 5.3.1.9); PFK, phosphofructokinase (EC 2.7.1.11); Ald, aldolase (EC 4.1.2.7); TIM, triose phosphate isomerase (EC 5.3.1.1); GAPD, glyceraldehydephosphate dehydrogenase (EC 1.2.1.12); PGK, phosphoglycerate kinase (EC 2.7.2.3); PGM, phosphoglyceromutase (EC 2.7.5.3); Enol, enolase (EC 4.2.1.11); PK, pyruvate kinase (EC 2.7.1.40); LDH, lactate dehydrogenase (EC 1.1.1.28); $P_2GM$, bisphosphoglycerate-mutase (EC 2.7.5.3); $P_2Gase$, 2,3-bisphosphoglycerate phosphatase (EC 3.1.3.13).

point it is appropriate to consider briefly some of the characteristics of biological systems.

1. *Biological systems are hierarchical.* One may distinguish two types of hierarchy: one, a hierarchy with respect to the time scales, the characteristic time of which range over more than fifteen orders of magnitude. At the lower end are the elementary reactions of the enzymes, on the upper genetic regulations in evolution. Secondly, there is a hierarchy of material structures, ranging from low-molecular compounds to organisms and populations. It is obviously a hopeless task to encompass the totality of all levels of time and structural organization. Modeling of metabolism singles out a range of about 5–6 orders of magnitude from $10^{-2}$ to $10^{4}$ seconds and limits itself essentially to the cell and its substructures.
2. *Metabolism is subdivided into functional units.* Such units are the metabolic pathways of the cell. What constitutes a metabolic pathway? An obvious answer is derived from the fundamental goal of the study of intermediary metabolism to elucidate the fate of any substrate from its entry into a cell to its final destination. Metabolic pathways may be considered to be semiindependent functional entities. This view is supported by comparative studies which show differences in types and quantitative relations of pathways among the organs of one species as well as among different species. Pathways tend also to react differently to changes of the functional state of the organism. On the other hand, groups of pathways are interconnected both functionally and by connecting metabolites and thus form complex subsystems of the cell. For instance, one may subsume all ATP-producing pathways as the energy-producing subsystem. Functionally, neither glycolysis nor respiration are fully independent since they react vicariously to the energy needs of the cell and are strongly interacting with each other.

   Despite the apparent clarity of the concept of metabolic pathways the cellular physiologist and even more so the modeler frequently encounter difficulties in the unambiguous definition and confinement of subsystems of metabolism, which could be investigated in isolation experimentally or theoretically. A definition which would meet the stringent theoretical demands on a subsystem of the cell would require that each component of the isolated subsystem is formed and degraded by reactions which are part of the model considered. The exceptions permitted are storage end-products such as glycogen and metabolites which change so slowly that they may be taken as constant for the time period investigated. According to such a definition of a system the oxidative pentose phosphate pathway, for instance, does not constitute an independent metabolic pathway. Furthermore, there may be substrates of pathways which are saturating and need not be considered for the dynamical description of the system.
3. *Biological systems are regulated.* Their regulatory properties are expressed in the precise determination of kind, amount, distribution in space and kinetics of most of their components. Regulation is characterized by the teleonomic response of biological systems to external and internal signals. There exist identifiable elements which cause changes of the behaviour of biological systems, i.e. controls. There exist common regulatory principles such as feedback inhibition, buffering systems for metabolites and off- and on- switching of complementary processes.

The investigation of the regulation of metabolic systems includes the answering of the following questions: How does a metabolic pathway react to an external signal? Which enzymes are important for the control of the flux and the metabolites? Where and how do external signals interact with an important enzyme? By what mechanism are different pathways which form a subsystem interconnected?

## 2. *Why Modeling?*

Modeling is an advanced technique for the study of the regulation. It involves the quantitative consideration of the multitude of data and interactions characteristic for biological systems. Models are simplifying abstractions of the reality. They may be

useful for the deduction of the essential relations in metabolism which constitute a characteristic dynamic structure. If the model is compatible with the experimental data it is likely that the dynamic structure of the system is correctly assumed. Many non-essential interactions can therefore be excluded as non-regulatory. Since the structure of the model is less complex than that of a real system and since the model is manipulable the basic principles of regulations may be evaluated. The model may be regarded as a hypothesis which is tested by the data. Usually, there is a choice among several hypothesis. Modeling may reject some of them but cannot positively prove any of them. However, modeling may help to design further experiments which discriminate better between alternatives. In many cases the model is not compatible with the data at the beginning although the incorporated knowledge seemed to be established experimentally. Thus, the simulator may discover inconsistencies in the experimental data or establish a new hypothesis (e.g. Garfinkel, 1971). The model allows for a coherent representation of the data and avoids the empirical accumulation of a multitude of facts which sometimes are irrelevant. Finally, modeling has a practical aspect. It can be hoped, that the behaviour of the system may be predicted or even designed so that the cells can be manipulated in the desired way (Garfinkel, 1976; Garfinkel *et al.*, 1976).

### 3. *Preconditions of Modeling*

The most important precondition is the knowledge of the main metabolic routes. It requires the identification of the stoichiometric relations in the various enzymatic reactions, branching and merging points, bypasses and most of the intermediates. The enzymes should have been identified and characterized kinetically so that the main effectors of their activity are known. It is then possible to supplement the stoichiometric network by addition of the non-stoichiometric interactions by connecting the metabolites with their target enzymes which they affect allosterically. Simplified rate laws of the enzymes and the $K_m$ and $K_i$-values should be known. Compartmentation of metabolites have to be recognized. It is obvious that not only their effective concentrations in each compartment but also the transport processes between compartments should be known. For mitochondria information on these points is now available (Gumaa *et al.*, 1971). Enzyme–enzyme-interactions although an interesting object of study appear to be of less importance. An important step toward the understanding of the regulation of metabolism and an important precondition for modeling is the elucidation of the flux distribution in the network. This is usually performed by isotope techniques in one of two possible ways, which are both applied to systems in metabolic steady states. In the first method the isotopic steady state is used and one calculates the flux distribution from the appearance of labelled end-products or from the loss of label in certain positions of an intermediate (e.g. Katz and Rognstad, 1967; Raugi *et al.*, 1975; Reich *et al.*, 1968; Katz and Rognstad, 1976). The second method is based on the time dependent appearance of label in the various metabolites (e.g. Garfinkel, 1970). It allows not only the estimation of flux-distributions but also of pool sizes, precursor–product-relations and of the number of isotopic compartments of a metabolite. Whereas the first method is a fit of an algebraic system of equations to the data, the second one uses linear differential equations. Although both procedures are often carried out by computer one should distinguish them clearly from modeling. The flux distribution through different pathways in a steady state network does not tell anything about regulatory enzymes, nor about regulatory principles, such as feedback interactions, nor about the expected change of the flux distribution after the action of an effector. Thus, the isotope techniques can identify the state of a steady state system, but cannot predict or explain it. On the other hand the building of a realistic model is greatly facilitated by knowledge of the magnitudes of branching and merging pathways provided by the isotopic method.

An essential precondition for the elucidation of regulatory principles is the study of the system in different states. One may deal with physiological or pathological states such as starvation, refeeding or diabetes. The different states of a system can also be brought about by pH-shifts, addition of hormones or other effectors. It is obvious that

modeling has no sense if the concentrations of the metabolites are known only for a single state of the system.

### 4. *Different Kinds of Models*

There are two extremes. On the one hand, the model may be intended to give a minutely detailed representation of the biological system which includes the properties of every component regardless of its role. This approach provides a kind of "mathematical photography" of the system. The purpose is primarily a close fit of the data. It permits the performance of simulated experiments which may save the experimenter considerable time and effort. The weakness of this kind of modeling is its empirical nature. Since no reduction of variables is intended the model is still almost as complex as the real system. The essential relations are therefore difficult to extract. The other extreme constitute idealized skeleton models which are intended to represent basic features of a biological system without direct confrontation with experimental data. Usually, there are alternatives and therefore the results cannot be directly applied to the actual biological system. Of course there are compromises between the two extremes of modeling by the use of models which are as simple as compatible with the real system.

The simplicity of a model can be achieved by reduction of the number of variables or parameters. The method most widely used, which is also soundly founded mathematically, is model reduction by the use of the time hierarchy of the system (see Section III.5). Only the essential dynamic variables have to be considered at the time level chosen since the faster variables are in a quasi-steady state and slower ones are constant. The application of this reduction is not an oversimplification. The error involved can be estimated and is the smaller the more pronounced the time hierarchy. Another principle of model reduction is based on the topological contraction of metabolic pathways. Adjacent reactions may be lumped into an overall reaction. Whole pathways may be substituted by single reactions in this way. For instance, Selkov (1975a) considered both the hexokinase and phosphofructokinase as a single phosphorylating reaction and the phosphoglycerate kinase and pyruvate kinase reaction as one reaction regenerating ATP. The principles of topological contraction have not been investigated in detail. The method has to be applied cautiously until the conditions under which the dynamics of the system is conserved have been established. A third way of reduction is based on the use of descriptive rate laws for the individual enzymes. Figure 1 shows three typical shapes of the dependence of enzymatic velocity on substrate concentration. Although the underlying physical mechanism may be very complex only a few parameters suffice for the representation of the entire information content of the curves. For instance, the use of the Hill-equation (Fig. 1c) requires fewer parameters than the model of Monod *et al.* (1965). Linearization of rate laws with respect to the variables is a special simplification (Heinrich and Rapoport, 1974a). Its use is confined to the range of low substrate concentrations and requires prior checking of its justification.

### 5. *Methods of Modeling*

In this section the attempt is made to classify models from the point of view of methods used. Such a classification is, of course, somewhat ambiguous and does not necessarily represent faithfully the intentions of their originators. An earlier review of modeling has been given by Garfinkel *et al.* (1970) which was grouped according to biological objects.

#### (a) *Data Description*

This kind of modeling is a test of consistency of data and of an underlying mathematical structure. There exist two possibilities of adjustment. First, the mathematical structure, i.e. the model itself, can be changed and secondly, the parameters of a chosen model can be varied. A model can only be rejected if no satisfactory fit with the data can be obtained for any of the possible parameter sets. As the number of parameters increases it becomes more and more difficult to get a unique best set.

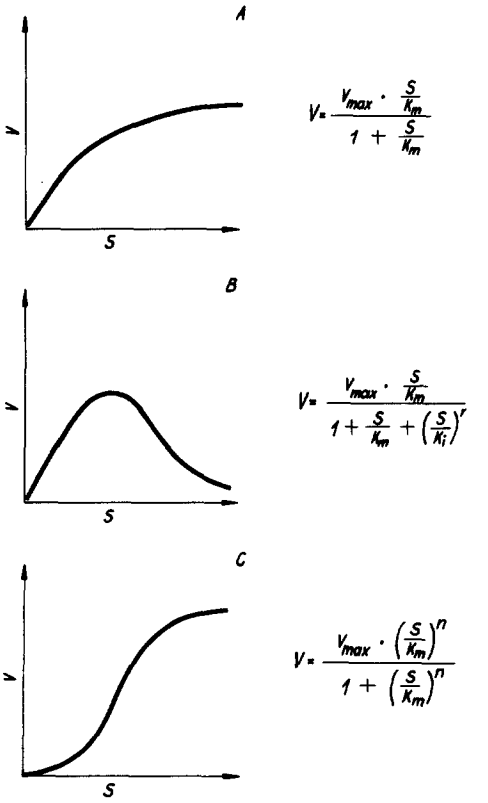



FIG. 1. Typical rate functions of enzymes. Three typical shapes of the dependence of the enzyme velocity on the substrate concentration are shown in schematical way. The curves can be fitted in most cases by the simple expressions given in the figure. A: Michaelis–Menten equation. B: Substrate inhibition. C: Hill equation.

The comparison of the behaviour of the model with the data can be either qualitative or quantitative. The qualitative comparison is based on the similarity of curves or on certain effects which the model can reproduce. Qualitative comparisons have been used in many studies on multienzyme systems (London, 1966; Selkov, 1968; Wright *et al.*, 1968; Higgins, 1973; Alberghina, 1974; Kothe *et al.*, 1975). The quantitative comparison is based on a goodness-of-fit criterion, for instance on the sum of least squares. This method is now generally applied in modeling of the kinetics of single enzymes (see Wong, 1975).

A model gains in validity the more data of different kind it describes including the metabolites and the enzyme parameters. It is strengthened if it is based on as many different conditions as possible. The fitting of a steady state model is a weaker criterion than that of a model of time dependent processes since in the latter case many more data points have to be described with the same number of adjustable parameters. Table 1 outlines the different levels of data description that will be considered.

(i) *Single Enzymes.* A survey of models of the steady state kinetics of single enzymes is beyond the framework of this review. Most of the problems have been solved such as discrimination between alternative models, parameter estimation, goodness-of-fit criteria as well as the information content of experimental data and the choice of an appropriate model (Cleland, 1967; Haarhoff, 1969; Reich, 1970; Bartfai and Mannervik, 1972; Reich *et al.*, 1972; Ottaway, 1973; Endrenyi and Kwong, 1973; Reich *et al.*, 1974; Kurganov *et al.*, 1974; Endrenyi, 1974; Reich and Zinke, 1974; Atkins, 1976; Markus *et al.*, 1976). It is generally recommended that for model selection graphical or simple computer diagnostica should be used, and non-linear regression analysis for parameter estimation. The modeling of single enzymes can be done more rigorously than that of multienzyme systems. The reasons for this rigor are that (a) only few parameters

TABLE 1. DIFFERENT LEVELS OF DATA DESCRIPTION

| | Single enzyme | Comparison of rate laws *in vitro* and *in vivo* | Prediction of steady state metabolites | Prediction of time dependent changes |
|---|---|---|---|---|
| Given quantities | substrate and effector concentrations in the test tube | Rate law *in vitro* and parameter values | Rate laws of all non-equilibrium enzymes and parameter values | Rate laws of all non-equilibrium enzymes and parameter values |
| Measured quantities | velocity of the enzyme | flux through the enzyme and some metabolite concentrations *in vivo* | all fluxes and metabolite concentrations in the system | all fluxes and metabolite concentrations in the system |
| Results of data description | rate law *in vitro* and parameter values | rate law *in vivo* | steady state "rate law" of the whole system | dynamics of the system |

are involved in many models, (b) the precision of the data is much greater, (c) physical–chemical methods provide for independent information concerning reaction pathways and parameter values, (d) the data structure is more appropriate for discrimination and (e) only one response variable, i.e. the reaction velocity has to be fitted to the data. Nevertheless, even for single enzymes there is much doubt that kinetic models in general reflect the actual physical process. However, as discussed in Section III.5 the detailed kinetic mechanisms of many enzymes (equilibrium enzymes) is of no importance either for the regulation in the cell or for modeling purposes.

(ii) *Comparison of Rate Laws and Enzymes* in vitro *and* in vivo. Often the purpose of the kinetic modeling of single enzymes is the application to the conditions in the cell. Here different levels may be distinguished. The lowest level is a mere comparison of the metabolite concentrations found in the cell with the corresponding $K_m$- or $K_i$-values of the enzymes. This leads usually to the distinction between important and unimportant interactions. The rate laws applicable to the conditions *in vivo* are therefore often simpler than those found in the test tube (e.g. Gerber *et al.*, 1974). It is obvious that kinetic studies with this purpose should be performed under conditions closely resembling those in the cell (e.g. pH, ionic strength). A difficulty often encountered in the transfer of *in vitro* results to the conditions *in vivo* are the low enzyme concentrations usually employed *in vitro*. Dissociation–association phenomena which are concentration dependent can alter the kinetics of enzymes such as phosphofructokinase (Frieden and Colman, 1967; Hofmann, 1976). The use of permeabilized cells seems to be a promising approach to measure the enzyme velocities *in situ* under conditions of high protein concentrations while low-molecular effectors can be changed at will (Sols and Marco, 1970; Reeves and Sols, 1973).

Some enzymes have very complex kinetics and are influenced by many effectors. Usually, the simultaneous action of these effectors has not been studied *in vitro*. Difficulties arise with enzymes in compartments (e.g. mitochondria) or in membranes. For compartmentalized enzymes the metabolite concentrations in the compartment have to be used (Gumaa *et al.*, 1971). A higher level of testing is that of the consistency between rate laws *in vitro* and *in vivo* as provided by the measurement of the velocities of the enzymes and the concentrations of substrates and effectors *in vivo* (e.g. Barwell and Hess, 1972; Wurster and Schneider, 1970; Bücher and Sies, 1969; Reeves and Sols, 1973).

For transitions from one steady state to another the analysis is often performed by means of the crossover theorem (see Section IV.3.(b)). Discrepancies may indicate the

additional action of effectors, compartmentation of metabolites or wrong assumptions in the *in vitro* rate laws. Sometimes it is possible to guess which is the additional effector by correlation analysis of the velocity of an enzyme and changes of the metabolites (Dietzler *et al.*, 1975a,b; Garfinkel *et al.*, 1968). Although it should be possible to estimate the best parameter values by computer optimization this has rarely be done (but see Garfinkel *et al.*, 1976). One reason is the low accuracy of the values for flux and metabolites *in vivo*, another the small number of experimental points. Oscillating metabolic systems seem to be favorable for the deduction of *in vivo* rate laws of enzymes (Giersch *et al.*, 1975). They allow for averaging over several cycles and for the calculation of velocities from the slopes of the metabolite changes versus time. An important and difficult question is the weighting of different data. The *in vitro* data on the velocities of isolated enzymes are usually quite precise whereas the deduced parameter values in the rate laws are not equally reliable. There are also great differences in the reliability of the determinations of the metabolite concentrations in the cell. Therefore appropriate decisions have to be made whether more confidence should be ascribed to the parameters in the rate laws or the *in vivo* metabolite concentrations. Some metabolites occur in very low concentrations or are so labile (e.g. 1,3-bis-phosphoglycerate) that large measurement errors are possible. In such cases it is therefore preferable to give more credence to the rate law and to adjust the concentrations of the metabolites. On the other hand, there are metabolites which can be precisely measured whereas the rate law of the enzyme is known to be oversimplified (e.g. PFK-reaction). In such cases the parameters rather than the metabolites should be adjusted. Both procedures of data correction have been used in an appropriate manner by Achs *et al.* (1971). However, since data correction is somewhat arbitrary it should only be done if the same correction may be applied in a number of experiments and if it is plausible to the experimenter. If different kinds of corrections are needed for every experiment the model is probably wrong.

Suitable apparent constants are often needed if the model should maintain simplicity (Rapoport *et al.*, 1976a; Garfinkel *et al.*, 1968). This includes apparent equilibrium constants which result from the neglect of hydratation, complex formation of reaction partners or the disregard of invariant participants.

(iii) *Prediction of the Metabolite Concentrations in the Steady State from the Kinetics of Individual Enzymes.* The task to be dealt with is the fit of a model based on individual enzymes with all the metabolites. The aim on this level of data fitting is a representation of the whole system. Two possibilities exist for the incorporation of the kinetics of single enzymes into a model of the system. In the first method the rate expressions of the elementary steps of an enzymatic reaction are used which describe the formation and degradation of all enzyme species considered. In the second procedure steady state rate laws for the enzymes are used instead (rate law method). In this manner rational functions appear in the mathematical model of the whole system. Whereas the first method may have the advantage that it is applicable under all circumstances and even for short time periods, it has both theoretical and methodical drawbacks. The detailed kinetics of the enzymes is usually unknown so that the enzyme mechanism contains hypothetical simplifications. The incorporation of the very low concentrations of enzyme species poses difficulties in the numerical solutions of the differential equations (the differential equations are "stiff"; see Section III.5). The rate law method is based on the time hierarchical structure of metabolism and has been shown to give results identical with the explicit method in its application to real systems (Vergonet and Berendson, 1970).

The fitting of the model of a multienzyme system requires the adjustment of the enzyme parameters to the steady state concentrations of the metabolites. This can be done by the sequential adjustment of the parameters of individual enzymes (Garfinkel *et al.*, 1968; Garfinkel and Hess, 1964). However, the information content of the metabolite concentrations in the steady state does not suffice to fix all the parameter values of the model. Some parameters are strongly correlated so that the effect of an increase

of the $V_{max}$-value of one enzyme on its product can be compensated by an increase in the $V_{max}$-value of the subsequent enzyme. It is therefore not certain that the model can predict the changes to a new steady state. Furthermore, the possibility of multiple steady states with different stability properties has to be considered. The individual adjustment of enzymes to the *in vivo* data does not yield the entire set of the steady state solutions of the system and the independent variation of the parameters of different enzymes is preferable. The prediction of the global steady state of a system is not only a test for the rate laws of the individual enzymes but also for the model structure (e.g. of the stoichiometric relations). A model which is capable of describing many steady states is a "rate law" of the whole system. However, compared to individual enzymes only a few different states are usually studied.

Rate laws for complex systems can also be obtained experimentally. Wilson *et al.* (1974) proposed a rate law for the respiratory chain which is very simple and might be used in a more complex model of metabolism as that of an overall reaction.

It has been argued that the results of the overall model may depend on the detailed rate laws of the constituent enzymes. Although no systematic study of this problem has been performed it has been repeatedly observed that the results do not depend on details of the kinetics. It has been shown for some cases that the assumption of random or ordered kinetics of an enzyme does not affect the overall behaviour of a model (Garfinkel, D., personal communication). *Ad hoc* rate laws have to be used if the enzymes have not been characterized sufficiently. These are usually simple first order rate laws or Michaelis–Menten functions (e.g. Wright and Gustafson, 1972; Rapoport *et al.*, 1976a). The same applies to overall rate laws which are employed for reactions which are catalyzed by several enzymes.

(iv) *Prediction of Time Dependent Metabolic Concentrations from the Kinetics of Individual Enzymes.* This is the most rigorous test of the model which is, however, performed so far in the least rigorous way. In contrast to the previous approach numerical solutions of differential equations must be fitted to the data rather than algebraic equations. Since the system of equations is complicated no intuitive prediction of the time dependent changes is possible and the simulations are a kind of "art" (Garfinkel, 1973) which requires skill and experience. Two methods introduced by Garfinkel can be used for data fitting. The first one is the variation of the rate law of an enzyme so that the influence of effectors on the flux may be correctly described. Owing to the circumstance that the fluxes are estimated from the accumulation of the products in some cases unreliable fluxes are calculated since one endproduct (e.g. lactate) may be present in large excess and can mask small changes. The fluxes may also be calculated from the measurements of the individual derivatives of the metabolite concentrations with respect to the time (Giersch *et al.*, 1975; Garfinkel *et al.*, 1976)

$$\frac{dS_i}{dt} = \frac{\Delta S_i}{\Delta t} = \sum_j v_{ij}. \tag{2.1}$$

The $v_{ij}$, the fluxes which form and degrade $S_i$, can be obtained in terms of $dS_i/dt$ as the solutions of a linear algebraic system of equations. However, if branches exist the number of fluxes may exceed that of metabolites and therefore not all fluxes can be determined. Of course, isotope techniques performed simultaneously with metabolic transitions are very suitable to calculate the fluxes (Stucki and Walter, 1972). The second method for data fitting (Achs and Garfinkel, 1968) starts with an important enzyme (e.g. phosphofructokinase) and uses initially a simple arbitrarily chosen rate law for the removal of products. The time dependent changes of the substrates and effectors are fed into the computer and the mechanism is varied until the products yield the desired time function. Then the enzyme mechanism is fixed and the adjacent enzyme is modeled until the intermediates give the right response. This procedure is continued until one obtains rate laws for all enzymes which lead to the expected behaviour of all metabolites.

Both procedures are purely empirical and lead only to non-unique solutions since the mechanism and parameters of only one enzyme are changed while all others are held fixed. The time dependent changes of a metabolite are considered as a function of only few enzymes, usually those preceding and succeeding it, whereas the dynamics of the metabolite could be the result of the complex interactions in the whole system.

A general procedure which in principle is able to lead to unique solutions is to integrate the whole system of differential equations in an iterative manner by trial and error. This procedure has been extended for automatic parameter estimation (Hemker, 1972; Curtis and Chance, 1972; Curtis, 1976) but seemingly has not been applied so far to a concrete case presumably because of the long computer time needed for the many integration steps. It has also been proposed to use hybrid computers so that the analog computer does the integration and the digital computer does the parameter optimization (Gibson and Parkhurst, 1968).

It is not easy to reject a model. Qualitative discrepancies which violate logic consistency such as incompatible changes of metabolites and fluxes are more important than quantitative differences (see Section IV.3.(b)). In some models a weak point is the practice to fix some metabolites at certain values or to introduce time dependent metabolites or time dependent parameters from the beginning (Park and Wright, 1975). Such given input functions constitute the boundaries of the model; their use limits the description to a subsystem. The introduction of time dependent input functions is justified if they are independent of the internal state of the system or so slow that they can be regarded as slow drift parameters. The use of time dependent input functions indicates that the dynamics of the system is not solely explained by its internal motions. The question arises whether the model has not been confined too narrowly.

(b) *Analysis of the Properties of a Model*

The present section deals with the next step of abstraction, i.e. with the investigation of the properties of a model. One general procedure involves the variation of the parameters of the model. The parameters which can be changed are not only enzyme constants such as $K_m$- and $V_{max}$ but also conservation quantities and input functions. By means of the sensitivity analysis (Anderson *et al.*, 1971) the influence of small (differential) changes of parameters is investigated. This allows also for the calculation of the control matrix and of control strengths (Section IV.2.(c)). The essential information obtained is the identification of the enzymes which have the strongest influence on steady state flux and metabolite concentrations. In time dependent processes the differential change of a parameter permits the identification of the time limiting enzymes, i.e. of those which influence strongly the transition time between two steady states (Section IV.4). Despite the fact that differential changes may not correspond to physiological relevant states they permit the clarification of the properties of the model. Non-differential changes are appropriate to predict the consequences of enzyme deficiencies or overproductions (Wright and Gustafson, 1972; Wright and Park, 1975; Rödenbeck *et al.*, 1975; Kothe *et al.*, 1975; Rapoport *et al.*, 1976a).

Conclusions may be drawn with respect to the regulation of a system from the analysis of the changes of the parameters necessary for a satisfactory description of the experimental data (e.g. blood preservation conditions, Rapoport and Heinrich, 1975). Parameter changes can be useful for the comparison of pathways in different animal species and may lead to the elucidation of evolutionary adaptation mechanisms (Rapoport *et al.*, 1976a; see Section V.1.(b)).

The change of conservation quantities, e.g. that of the adenine nucleotides may show the consequences of a limitation of pools.

Nonlinear differential equations possess a great manifold of solutions and the character of the solutions depends strongly on the choice of the parameter values. Thus, the trajectories of a system which originally converged to a stable steady state may display a limit cycle in response to a variation of a parameter (Section III.4). In most

cases it is necessary to vary systematically the parameters to see which possibilities for the behaviour of a system exist.

One may also change the structure of a model, e.g. by alteration of enzyme mechanisms, consideration of additional pathways or enzymatic reactions, incorporation of further feedback interactions, etc. This procedure allows, for instance, the elucidation of the role of pathways or of interactions between effectors and enzymes. By variation of the model structure its essential invariant features become apparent. It is also useful for extensions of the model to pathways in other biological systems.

Both structural and parameter changes of the model serve to analyse the space around the solution which has been fitted to the data. They are essential for the elucidation of the regulatory principles of a metabolic system, which would be difficult to obtain without mathematical models since the variation of parameters or even of the structure in a real biological system is possible only to a limited extent.

(c) *Idealized "Skeleton" Models*

An idealized model serves the aim to represent in the simplest manner the dynamic features of a system. Idealized models also serve as an object of detailed mathematical investigation since they are less intractable than detailed ones. The essential feature which is modeled may be, for example, the occurrence of oscillating behaviour of the metabolites (Higgins, 1964; Selkov, 1968; Goldbeter and Lefever, 1972), the constancy of the ATP-level under load (Selkov, 1975a,b; Reich *et al.*, 1976) or switching on and switching off of complementary pathways (e.g. Pasteur effect, glycolysis–glycogenolysis; Heinrich, 1976). The idealized models are not confronted directly with experimental data but represent abstractions of a large body of observations and experiments.

Since such models cannot be easily tested by experimental data the main criteria for their acceptance are biological plausibility and their ability to simulate teleonomical behaviour. There are two possibilities to arrive at a skeleton model. First, the idealized model may be extracted from a detailed one which was able to describe actual experimental data. The simplification can either be arrived at by the use of the time hierarchy or by topological contraction. A second approach which may be considered the reverse of the preceding one is to set up an idealized model as a preliminary step to a more detailed description. It is particularly appropriate if few details such as rate laws and parameter values of a metabolic system are known. In such a case one uses as a background the stoichiometric map, on the basis of which a model with shortened pathways and arbitrary rate laws is set up. Such a model may clarify the understanding of the regulation of a system and may pinpoint the areas of further experimentation. It may also be used to test the intuitive description of metabolic events.

There are, however, serious limitations to the usefulness of idealized models. They stem mostly from the lack of data fitting. Even if the model describes correctly the dynamics of a biological system the parameters used may not correspond to reality. Furthermore the models are not unique despite the fact that they contain in general few parameters. Comparison with experimental data is a much more stringent criterion for the correctness of a model. There are attempts to do so with respect to glycolytic oscillations (Higgins *et al.*, 1973; Richter *et al.*, 1975). Finally, although it is tempting to regard the idealized models as general representations of pathways of various biological systems, such an assumption has to be qualified on account of a highly specialized nature of the metabolism of some types of cells.

## III. MATHEMATICAL DESCRIPTION OF THE DYNAMICS OF METABOLIC SYSTEMS

### 1. *The Differential Equations*

The mathematical description of the dynamics of metabolic systems is performed by means of a system of differential equations derived from a consideration of the

fluxes which produce or remove the metabolites

$$\frac{dS_i}{dt} = \sum_{j=1}^{r} c_{ij} v_j \qquad (i = 1, \ldots, n). \tag{3.1}$$

Here $S_i$ signify the concentrations of the metabolites, $n$ their number, $v_j$ the activities of the enzymes participating in the metabolic pathway analyzed, $t$ the time and $r$ the number of the reactions in the system. The coefficients $c_{ij}$ are the elements of the *stoichiometric matrix*. They indicate which fluxes influence the metabolites. An element $c_{ij}$ is positive if the metabolite $S_i$ is the product of reaction $v_j$ and negative if it is the substrate, otherwise it is zero. The stoichiometric matrix contains therefore the information on the structural network, i.e. the "topology" of the enzyme system. The validity of the differential equations depends on the assumption that the metabolites may be considered to be equally distributed in the cellular space. It means that effects are not considered such as diffusion processes resulting from space dependencies in the metabolite concentrations. Secondly, it is presupposed, that the enzyme activities obey steady state rate laws, i.e. that all enzyme–metabolite complexes are considered to be in the steady state, so that they do not enter explicitly in the dynamical description of the enzyme system. It will be shown in Section III.5.(b).(ii) that this approximation is justified if the concentrations of the intermediate metabolite complexes are very small as compared with the concentrations of the metabolites. In this case the catalyzed reactions obey the following rate equations

$$v_j = v_j^+ - v_j^- = \left( p_j \prod_{\substack{i \\ \text{substrates}}} S_i - p_{-j} \prod_{\substack{i \\ \text{products}}} S_i \right) R_j(S_i, p_k). \tag{3.2}$$

The multiplication is performed in the first term over all substrates and in the second term over all products of the enzyme $E_j$. The fluxes depend not only on the concentrations of the metabolites but also on the kinetic parameters $p_k$, such as $K_m$-values of substrates and products and inhibition or activation constants of effectors. The kinetic constants $p_j$ and $p_{-j}$ are proportional to the concentration of the enzyme $E_j$ and indirectly proportional to the $K_m$-values of the reactants. The factor $R_j(S_i, p_k)$ contains all the information about the special kinetic properties of the enzyme. It will be called the *regulating factor*. In general it will depend not only on the concentrations of the immediate partners of the reaction but also on the concentration of other metabolites which act as effectors of the enzyme. The structure of the regulating factor may differ widely for various enzymes of one metabolic pathway.
By use of the *equilibrium constant* $q_j$ and the *mass action ratio* $\Gamma_j$,

$$q_j = \frac{k_j}{k_{-j}}, \quad \Gamma_j = \frac{\prod_{\substack{i \\ \text{products}}} S_i}{\prod_{\substack{i \\ \text{substrates}}} S_i} \tag{3.3}$$

the rate Eqn. (3.2) may also be written in the following form

$$v_j = k_j \prod_{\substack{i \\ \text{substrates}}} S_i \left( 1 - \frac{\Gamma_j}{q_j} \right) R_j. \tag{3.4}$$

Since at equilibrium $v_j = 0$, the mass action ratio in this case does not depend on the regulating factor. This has important consequences for the mathematical description of enzyme pathways that involve very fast reactions the reactants of which are near to equilibrium. The special kinetic mechanism of such enzymes is of no importance for the regulation of the pathway (see Section III.5).

Equation (3.1) constitutes a system of ordinary first order differential equations in which the fluxes depend on the metabolite concentrations and a set of kinetic parameters. Generally we shall write it in the following abbreviated manner,

$$\frac{dS_i}{dt} = f_i(S_j, p_k) = f_i^+ - f_i^-, \quad \begin{matrix}(i,j = 1, \ldots, n)\\ (k = 1, \ldots, m)\end{matrix} \tag{3.5}$$

where $m$ is the number of the kinetic parameters. $f_i^+$ summarizes all fluxes which produce the metabolite $S_i$ (inward flux) and $f_i^-$ all fluxes which remove it (outward flux). $f_i$ is called the net production rate of the metabolite $S_i$. In the case that the enzyme concentrations and the kinetic parameters are invariant with time the right side of Eqn. (3.5) does not depend explicitly on time. Such equations are called autonomous.

The state of a system at a time $t$ is defined by the metabolite concentrations at that time which may be described in a compact manner by the vector $\mathbf{S}(t) = (S_1(t), \ldots, S_n(t))$. The totality of possible states form the $n$-dimensional *state space* $R^n$. If the parameter values are fixed the solutions $S_i(t)$ form curves in the state space, the so-called trajectories which are functions of the initial values $S_i(t_0)$. In the case that the system contains only two metabolites $S_1$ and $S_2$ the state space degenerates to a *phase plane* $R^2$. The totality of all parameter values $(p_1, \ldots, p_m)$ form the $m$-dimensional *parameter* or *control space* $P^m$.

The rate equations are in general non-linear functions of the metabolite concentrations $S_i$ and the parameters $p_k$. With reactions involving more than one molecule the non-linearity is a direct consequence of the law of mass action. Therefore, the differential Eqns. (3.5) become also nonlinear and generally, their solutions cannot be given in a closed analytical form. There are three ways to deal with such equations

(1) to limit their solution to cases in which linear approximations are applicable
(2) to limit oneself to qualitative statements on the dynamic behaviour of the system
(3) numerical integration with specified parameter and initial values.

A survey of the steady state equations which are commonly employed in enzyme kinetics shows that the regulating factors always constitute a special type of function, namely ratios between two polynomials in the metabolite concentrations (Wong, 1975). This fact can be used to some advantage for the mathematical treatment of Eqns. (3.5), especially for time independent problems, since the solution procedure may be reduced to the determination of the roots of polynomials.

In a closed metabolic system without material exchange with its surroundings the sum of all metabolite concentrations (mass/volume) is constant and the following equation applies

$$\frac{dt}{dt}\left(\sum_{i=1}^{n} S_i\right) = \sum_{j=1}^{r}\left(\sum_{i=1}^{n} c_{ij}\right)v_j = 0. \tag{3.6}$$

Since this equation is valid for all $v_j$ the stoichiometric matrix of the closed system must fulfil the condition

$$\sum_{i=1}^{n} c_{ij} = 0. \tag{3.7}$$

Although metabolism as a whole is an open system, conservation equations of the type of Eqn. (3.7) may hold in a subsystem for groups of metabolites, the total concentration of which does not change with time. The number of differential equations is reduced by the number of conservation quantities which exist in the system considered.

### 2. *Steady States and Stability*

Metabolic systems generally are in a steady state which is defined by constant values of the flux and of the metabolite concentrations. For all intermediates the outflux equals the influx so that the rate of their net production becomes zero. Thermodynamically,

steady states are defined by minimum production of entropy (Glansdorff and Prigogine, 1971). Obviously a steady state must be distinguished from that of an equilibrium without net flux which is only possible in a closed system.

The application of the steady state condition to the differential Eqns. (3.1) and (3.5) yields

$$\frac{dS_i}{dt} = 0 = f_i(S_1^0, \ldots, S_n^0; p_k) = \sum_{j=1}^{r} c_{ij} v_j, \tag{3.8}$$

which is an implicit equation system for the steady state concentrations $S_i^0$. Generally, this equation is non-linear and must be solved numerically. The solution of a system of non-linear equations may be non-unique, i.e. there exist several steady states at one set of parameters.

Steady states may be dynamically stable or unstable. General definitions of stability, first given by Ljapunov (1893) are rather complex. (For a general definition of stability see Hahn, 1967; Rosen, 1970.) We shall limit ourselves to the following two definitions

(a) a steady state $S_i^0$ is *locally asymptotically stable* if the system returns eventually ($t \to \infty$) to its steady state after a small perturbation ($S_i = S_i^0 + \sigma_i$).
(b) If all trajectories lead to the steady state $S_i^0$ from any kind of perturbation it is *globally asymptotically stable.* It should be noted that only the stable steady states of a model can describe steady states of a real biological system.

Generally it is difficult to assess the global stability of a system. It is much easier to decide whether it is locally asymptotically stable by the following method. One expands the right sides of the differential Eqns. (3.5) in the neighbourhood of the steady state in a Taylor series. Since only infinitesimal deviations from the steady state have to be considered only the linear terms are of interest:

$$\frac{d\sigma_i}{dt} = \sum_{k=1}^{n} \frac{\partial f_i}{\partial S_k} \sigma_k = \sum_{k=1}^{n} A_{ik} \sigma_k. \tag{3.9}$$

$A_{ik}$ is the so called *Jacobi matrix* of the system. The linear differential equations have the well known solution

$$\sigma_i(t) = \sum_{k=1}^{n} B_i^k \, e^{\lambda_k (t - t_0)} \tag{3.10}$$

where $t_0$ denotes the initial time of perturbation. $\lambda_k$ are the *eigenvalues* of the Jacobi-matrix. They are calculated from the *characteristic equation* of the system

$$\mathrm{Det}|A_{ik} - \lambda \delta_{ik}| = 0, \quad \delta_{ik} = \begin{cases} 0 & i \neq k \\ 1 & i = k \end{cases}, \tag{3.11}$$

which represents a polynomial of the $n$-th degree

$$a_n \lambda^n + a_{n-1} \lambda^{n-1} + \ldots + a_1 \lambda + a_0 = 0. \tag{3.12}$$

The coefficient $a_0$ is identical with the determinant of the Jacobi-matrix ($\mathrm{Det}|A_{ik}|$). If it is zero, at least one of the eigenvalues is also zero. This is the case if some of the Eqns. (3.9) are linearly dependent on each other. The coefficients $B_i^k$ are functions of the kinetic parameters of the enzymes and of the initial values $\sigma_i(t_0)$. A steady state is asymptotically stable if all the eigenvalues, i.e. the roots of the characteristic Eqn. (3.12) have only negative real parts. In this case Eqn. (3.10) contains only terms which subside with time and disappear at $t \to \infty$. If one or more of the solutions of the characteristic equation are zero, the system exhibits a critical behaviour. Statements on its stability can only be made on consideration of the quadratic or higher terms of the expansion of Eqn. (3.5). Since for the analysis of stability only the signs, not the exact values of the roots are of interest, the solution of the characteristic Eqn. (3.12) is not required. The signs may be easily derived from its coefficients $a_i$. Mostly

the criteria given by Hurwitz and Routh are applied to prove that the polynomial has only roots with negative real parts (see Hahn, 1967). Stability requires that the coefficients fulfil the following conditions:

*for two metabolites*:

$$a_2 > 0,\ a_1 > 0,\ a_0 > 0 \tag{3.13}$$

*for three metabolites*:

$$a_3 > 0,\ a_2 > 0,\ a_0 > 0,\ a_2a_1 - a_3a_0 > 0. \tag{3.14}$$

Generalized statements on the stability of steady states can be made only for special types of systems. For example, Hearon (1953) showed that closed systems consisting of first order reactions are stable and cannot exhibit damped oscillations, i.e. that all eigenvalues are real and negative. In pseudo first order systems damped oscillations may occur but the steady states are always stable (Higgins, 1967).

### 3. *Two-Component Systems*

In some cases the time dependent behaviour of a metabolic system may be sufficiently described by a mathematical model, which contains only two state variables (see Section III.5). In the following section we apply the methods explained in the preceding section to the analysis of such systems. They are described by the differential equations

$$\frac{dS_1}{dt} = f_1(S_1, S_2; p_k) \tag{3.15a}$$

$$\frac{dS_2}{dt} = f_2(S_1, S_2; p_k). \tag{3.15b}$$

The dynamical investigation of such systems is much simpler than those of higher degree since the motions can be analysed in a two-dimensional system of coordinates. By eliminating the time $t$ from Eqns. (3.15a,b) we obtain the differential equation

$$\frac{dS_2}{dS_1} = \frac{f_2(S_1, S_2; p_k)}{f_1(S_1, S_2; p_k)}, \tag{3.16}$$

the solution of which are the trajectories $S_2$ $(S_1)$ in the phase plane. For the qualitative discussion of the form of the trajectories two curves, $S_2^{\mathrm{I}}$ $(S_1)$ and $S_2^{\mathrm{II}}$ $(S_1)$, are of particular importance which are defined by the zeros of the numerator and denominator, respectively, of Eqn. (3.16).

$$f_1(S_1, S_2^{\mathrm{I}}) = 0,\quad f_2(S_1, S_2^{\mathrm{II}}) = 0. \tag{3.17}$$

The direction of the trajectories on the $S_2^{\mathrm{I}}$-curve are parallel to the $S_2$-axis and those on the $S_2^{\mathrm{II}}$-curve to the $S_1$-axis (see Fig. 2). The curves are called quasi-steady state lines; the steady state lies at their intersection.

Linearization of the Eqns. (3.15a,b) in the neighbourhood of a steady state yields for small perturbations $\sigma_1$ and $\sigma_2$

$$\frac{d\sigma_1}{dt} = \frac{\partial f_1}{\partial S_1}\sigma_1 + \frac{\partial f_1}{\partial S_2}\sigma_2 = a_{11}\sigma_1 + a_{12}\sigma_2 \tag{3.18a}$$

$$\frac{d\sigma_2}{dt} = \frac{\partial f_2}{\partial S_1}\sigma_1 + \frac{\partial f_2}{\partial S_2}\sigma_2 = a_{21}\sigma_1 + a_{22}\sigma_2. \tag{3.18b}$$

The eigenvalues of the Jacobi-matrix are determined by the equations

$$\lambda^2 - (a_{11} + a_{22})\lambda + (a_{11}a_{22} - a_{12}a_{21}) = 0, \tag{3.19}$$

which has the two solutions

$$\lambda_{1,2} = \frac{\mathrm{tr}}{2} \pm \sqrt{\frac{\mathrm{tr}^2}{4} - \Delta} \tag{3.20}$$

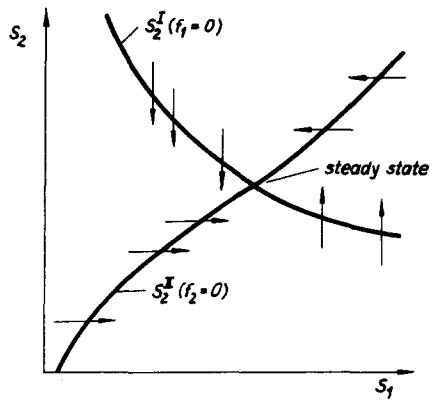



FIG. 2. Two-dimensional ($S_1$, $S_2$) phase plane. The arrows indicate the direction of the motion on the quasi-steady state lines $S_2^I(S_1)$ and $S_2^{II}(S_1)$.

where tr is the trace and $\Delta$ the determinant of the Jacobi-matrix:

$$\mathrm{tr} = a_{11} + a_{22}, \Delta = a_{11}a_{22} - a_{12}a_{21}. \tag{3.21}$$

The equation system (3.18) has the following solution

$$\sigma_1(t) = \frac{c_1 a_{12}}{\lambda_1 - a_{11}} e^{\lambda_1(t-t_0)} + c_2 e^{\lambda_2(t-t_0)} \tag{3.22a}$$

$$\sigma_2(t) = c_1 e^{\lambda_1(t-t_0)} + \frac{c_2 a_{21}}{\lambda_2 - a_{22}} e^{\lambda_2(t-t_0)}. \tag{3.22b}$$

For $t = t_0$ Eqns. (3.22a,b) become a linear system for the determination of the coefficients $c_1$ and $c_2$ from the initial perturbations $\sigma_1(t_0)$ and $\sigma_2(t_0)$.

It follows from Eqn. (3.20) that the signs of the eigenvalues and therefore the stability behaviour of the systems is defined uniquely by the two terms tr and $\Delta$ which are generally complex functions of all kinetic parameters. Figure 3 shows the plane defined by tr and $\Delta$. It is easily recognized that the real parts of both eigenvalues are negative only in the fourth quadrant, in which $\Delta > 0$ and $\mathrm{tr} < 0$. Only for parameter values for which the corresponding ($\Delta$, tr) values lie in this domain the corresponding motion is stable. The axes of the coordinates and the line

$$\mathrm{tr}^2 = 4\Delta \tag{3.23}$$

divide the ($\Delta$, tr) plane into six domains, in which the character of the eigenvalues,

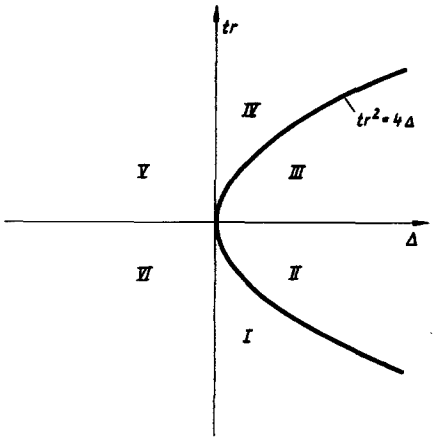



FIG. 3. Regions in the ($\Delta$, tr)-plane with different dynamic characteristics in the neighbourhood of the steady state. I: stable node; II: stable focus; III: unstable focus; IV: unstable node; V, VI: saddle point.

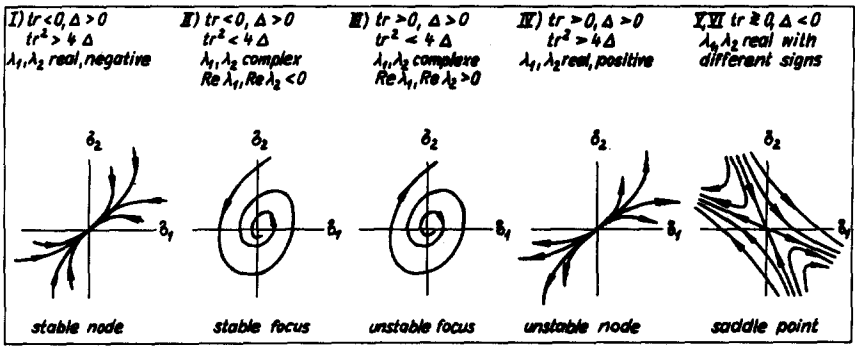


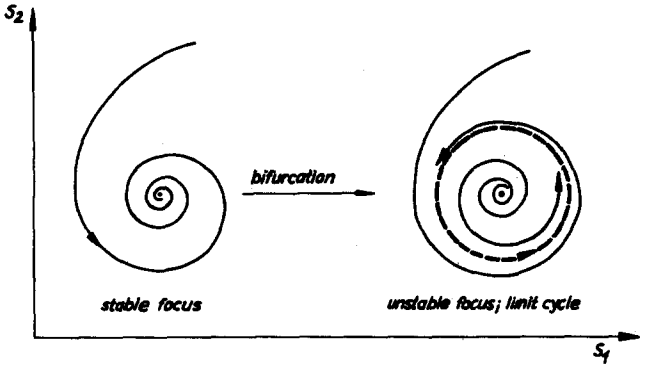

FIG. 4. Types of motion in the neighbourhood of steady states corresponding to the six domains of the (Δ, tr)-plane (see Fig. 3).

and therefore the type of the resulting motion, differ from each other. Figure 4 gives a summary of the possible types of motion.

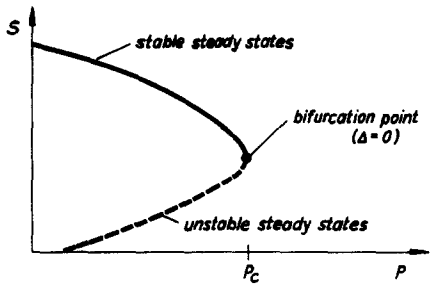
### 4. *Parameter Dependence of the Dynamics of Metabolic Systems; Bifurcations*

The variation of the values of the parameters yields both biological and mathematical insight in the properties of the model (see Section II.5.(b)). For the analysis of the parameter dependence of the model such combinations of parameters are of particular interest which produce changes of the stability characteristics of steady states. Such parameter combinations are called *bifurcation points.* At first we shall discuss the two-dimensional case. As pointed out in the foregoing section only the trace and the determinant of the Jacobi-matrix and not parameter values *per se* determine the stability of the system. It is therefore convenient to analyse the parameter changes not in the entire $m$-dimensional parameter space, but in the two-dimensional (Δ, tr)-plane. Changes of the parameters will produce a curve in the (Δ, tr)-plane. If it does not exceed the limits of the six domains described (see Fig. 3), the stability characteristics remains unaffected. This holds also for the case of the crossing of the dividing line defined by $\mathrm{tr}^2 = 4\Delta$ between the domains I and II and III and IV, respectively. There occurs merely a transition from a focus to a node (see Fig. 4), Qualitative changes may be expected if (1) the axis $\mathrm{tr} = 0 (\Delta > 0)$ or (2) the axis $\Delta = 0$ are crossed.

(1) In the first case (Hopf-bifurcation) the stability characteristic changes since the real parts of the eigenvalues change signs (see Eqn. (3.20)). If they become positive a stable focus becomes an unstable one (see Scheme 1). If there can be found in the phase plane a closed line $C$ around the unstable steady state which is transversed by all trajectories from outside it is clear that the variables can never reach constant values. Hopf proved in a classical paper that under such conditions the trajectories




Scheme 1.




Scheme 2.

merge to a closed trajectory which is called a *limit cycle* (Hopf, 1942). Moving on the limit cycle the system exhibits undamped oscillations. In a linear approximation which is valid for small amplitudes of this motion, the duration of the period $T$ of the oscillations is equal

$$T = \frac{2\pi}{\mathrm{Im}(\lambda)}. \tag{3.24}$$

$\mathrm{Im}(\lambda)$ represents the imaginary part of that eigenvalue the real part of which became positive at the bifurcation.

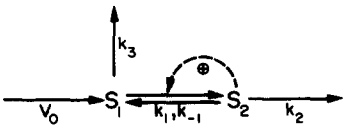
(2) In the second case, if the axis $\Delta = 0$ is crossed, the number of steady states changes. This behaviour is shown in Scheme 2. With increase of a parameter ($p$) a stable and an unstable state approach and cancel each other at a critical value ($p_c$) at which $\Delta = 0$.

Such considerations apply also for systems of higher dimensions with some modifications. The condition for the emerging or disappearance of pairs of steady states at $\Delta = 0$ ($\Delta = \mathrm{Det}|A_{ik}|$) remains unchanged. For the case of the Hopf bifurcation the condition $\mathrm{tr} = 0$ which indicates the change of the sign of an eigenvalue has to be replaced by more complicated conditions, e.g. the Routh criterion.

(a) *Simplified Biochemical Systems with Bifurcation Properties*

The theoretical considerations may be illustrated by two simplified biochemical examples. They show both the bifurcation of a stable steady state into multiple steady states as well as the emergence of an unstable steady state which is surrounded by a limit cycle.

(i) *Feedback Activation in a Two-Component System.* The essential feature of the system considered in this section is an enzyme which is activated by its product. The system originates from the study of oscillations in glycolysis (Higgins, 1964; Selkov, 1968, see also Section V.4). In the system of reactions, shown in Scheme 3 it is assumed that the enzyme which catalyze the reversible reaction $S_1 \rightleftarrows S_2$ is activated by the product $S_2$ and that all other enzymes catalyze irreversible reactions.




Scheme 3.

If one presupposes that all enzyme activities depend linearly on the substrate concentrations and that the input $v_0$ of the metabolite $S_1$ is constant one arrives at the following differential equations

$$\frac{dS_1}{dt} = v_0 - (k_1 S_1 - k_{-1} S_2)(1 + cS_2^q) - k_3 S_1 = f_1(S_1 S_2) \tag{3.25a}$$

$$\frac{dS_2}{dt} = (k_1 S_1 - k_{-1} S_2)(1 + cS_2^q) - k_2 S_2 = f_2(S_1, S_2), \tag{3.25b}$$

$c$ and $q$ are the activation parameters of $E_1$. It will be shown that this system may become unstable at certain parameter values and that it may have several steady states for one set of parameters.

For the quasi-steady state lines one obtains:

$$S_1^{\mathrm{I}} = \frac{v_0 + k_{-1} S_2 (1 + cS_2^q)}{k_1 (1 + cS_2^q) + k_3} \tag{3.26a}$$

$$S_1^{\mathrm{II}} = \frac{S_2 [k_2 + k_{-1}(1 + cS_2^q)]}{k_1 (1 + cS_2^q)}. \tag{3.26b}$$

Furthermore one gets by simple differentiation of the net production rate defined in Eqns. (3.25a,b)

$$\mathrm{tr} = -k_1 (1 + cS_2^q) - (k_{-1} + k_2 + k_3) - k_{-1} cS_2^q + \frac{k_2 cq S_2^q}{(1 + cS_2^q)}; \tag{3.27a}$$

$$\Delta = -\frac{k_2 k_3 cq S_2^q}{(1 + cS_2^q)} + k_3 k_{-1} cS_2^q + k_2 k_3 + k_3 k_{-1} + k_1 k_2 (1 + cS_2^q). \tag{3.27b}$$

By use of the steady state condition

$$S_1^{\mathrm{I}}(S_2) = S_1^{\mathrm{II}}(S_2) \tag{3.28}$$

the variable $S_2$ may be eliminated from Eqns. (3.27a,b) so that both tr and $\Delta$ can be expressed only as functions of the parameters $p_k$ of the system. After that, the equations $\mathrm{tr}\,(p_k) = 0$ $(\Delta > 0)$ or $\Delta(p_k) = 0$ which define the bifurcation points may be used to calculate the value of one parameter as a function of the others. Thus, one obtains for the activation parameter $c$ at $\mathrm{tr} = 0$

$$c_{1,2}^{\mathrm{tr}} = \frac{(a \pm b) k_2^q}{v_0^q} \left(1 + \frac{k_{-1} k_3}{k_1 k_2} + \frac{k_3}{k_1} (1 + a \pm b)^{-1}\right)^q \tag{3.29}$$

with

$$a = \frac{(q-1) k_2 - 2(k_1 + k_{-1}) - k_3}{2(k_1 + k_{-1})}, \quad b = \sqrt{a^2 - \frac{k_1 + k_{-1} + k_2 + k_3}{k_1 + k_{-1}}} \tag{3.30}$$

and, at $\Delta = 0$,

$$c_{1,2}^{\Delta} = \frac{(x \pm y) k_2^q}{v_0^q} \left(1 + \frac{k_{-1} k_3}{k_1 k_2} + \frac{k_3}{k_1} (1 + x \pm y)^{-1}\right)^q, \tag{3.31}$$

with

$$x = \frac{(q-1) k_2 k_3 - 2 k_{-1} k_3 - 2 k_1 k_2}{2(k_{-1} k_3 + k_1 k_2)}, \quad y = \sqrt{x^2 - \frac{k_{-1} k_3 + k_1 k_2 + k_2 k_3}{k_{-1} k_3 + k_1 k_2}}. \tag{3.32}$$

Since all kinetic parameters are real numbers, changes in the signs of tr and $\Delta$ can only occur if the values of $b$ and $y$ which are determined by Eqn. (3.30) and Eqn. (3.32), respectively are also real. Thus, from the condition that $b$ is real

$$a^2 > \frac{k_1 + k_{-1} + k_2 + k_3}{k_1 + k_{-1}}, \tag{3.33}$$

one obtains the inequality

$$q^{\mathrm{tr}} > 1 + 2\left(\frac{k_1 + k_{-1}}{2}\right)\left(\sqrt{1 + \frac{k_2 + k_3}{k_1 + k_{-1}}} + 1\right) + \frac{k_3}{k^2} = q_0^{\mathrm{tr}}, \tag{3.34}$$

and from the condition that $y$ is real

$$x^2 > \frac{k_{-1} k_3 + k_1 k_2 + k_2 k_3}{k_{-1} k_3 + k_1 k_2} \tag{3.35}$$

the inequality

$$q^{\Delta} > 1 + 2\left(\frac{k_1}{k_3} + \frac{k_{-1}}{k_2}\right)\left(\sqrt{1 + \frac{k_2 k_3}{k_{-1} k_3 + k_1 k_2}} - 1\right) = q_0^{\Delta}. \tag{3.36}$$

From the inequalities (3.34) and (3.36) it follows that the activation constant $q$ must exceed the values $q_0^{\mathrm{tr}}$ and $q_0^{\Delta}$ for the appearance of instabilities or multistationary states, respectively. The faster the enzyme

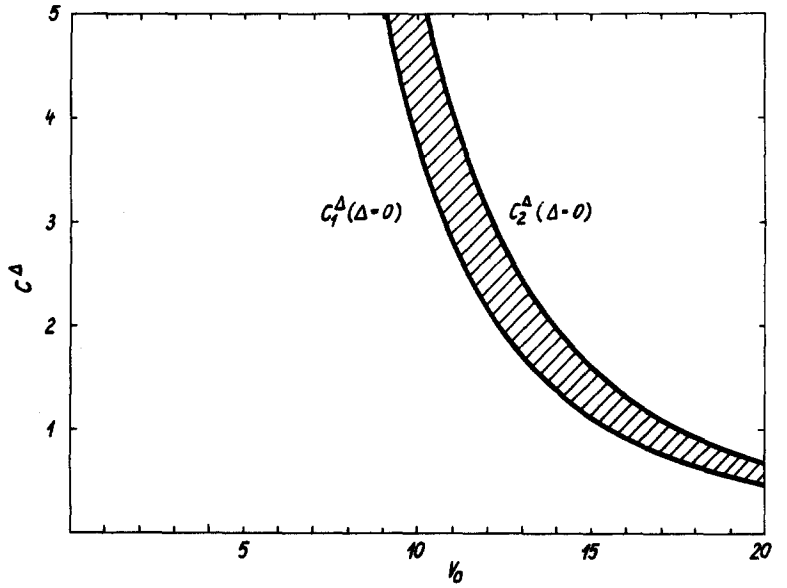



FIG. 5. Parameter regions with different stability properties of the reaction system represented in Scheme 3. Parameter values: $k_1 = 1$, $k_{-1} = 0$, $k_2 = 5$, $k_3 = 1$, $q = 3$. For parameter values $c$ and $v_0$ within the hatched area the steady states are unstable.

$E_1$ the greater must be the activation constant $q$. If the reaction catalyzed by $E_1$ is near equilibrium ($k_1, k_{-1} \gg k_2, k_3$) a unique stable steady state exists.

Figure 5 shows the lines $c_{1,2}^{tr}$ as functions of $v_0$ for parameter values $k_1$, for which inequality (3.34) but not (3.36) is satisfied. Multiple steady states may be excluded. On crossing into the domain bounded by $c_1^{tr}$ and $c_2^{tr}$ the steady state becomes unstable and a limit cycle arises.

Figure 6 shows in the ($S_1$, $S_2$)-phase plane the quasi-steady state lines $S_1^I$ and $S_2^{II}$ and numeric integrations of the differential equations (3.25*a,b*). In Fig. 6a the parameters were taken from the stable domain of the ($v_0$, $c$)-plane in Fig. 5. The trajectory is a spiral and approaches the steady state with time. In Fig. 6b the parameter values were taken from the unstable domain of the ($v_0$, $c$)-plane in Fig. 5. The trajectory approaches a limit cycle on which the system exhibits undamped oscillations.

Figure 7 shows the lines $c_{1,2}^{\Delta}$ as a function of $v_0$ for parameter values which only satisfy the inequality (3.36). Since for these lines $\Delta = 0$, the number of steady states changes by two when they are crossed. Within the domain enclosed by $c_1^{\Delta}$ and $c_2^{\Delta}$ there exist three steady states. Figure 8 shows the steady states $S_1^0$ and $S_2^0$ as a function of the input $v_0$ for different values of $c$ and with fixed parameter values $k_i$. In Fig. 9 is shown the behaviour of the steady states as a function of the input ($v_0$) at different values of $k_1$ and $k_{-1}$. It may be seen that with acceleration of the reversible reaction the steady state becomes unique.

For the special case of $q = 2$, $k_3$, $k_{-1} = 0$ the reaction mechanism may be written in the following manner

$$\begin{aligned} \textit{Input} &\xrightarrow{v_0} S_1 \\ S_1 &\xrightarrow{k_1} S_2 \\ S_1 + 2S_2 &\xrightarrow{bk_1} S_2 \\ S_2 &\xrightarrow{k_2} \textit{output}. \end{aligned}$$

It is equivalent to the reaction mechanism dealt with by Prigogine and Lefever (1968) and Tyson and Light (1973). They demonstrated that in a system of two components, which enter maximally trimolecular reactions only this type of limit cycle oscillator can exist.

(ii) *Feedback-Inhibition in an Unbranched Pathway.* Since the discovery of feedback-inhibition by endproducts in the biosynthetic pathways of amino acids (Umbarger, 1956; Gerhart and Pardee, 1962) it has been pointed out that this type of regulation is optimal for the stabilization of steady states of unbranched enzymatic systems (see, for example, Savageau, 1974). If the feedback inhibition is sufficiently strong the rate of synthesis of the end product becomes nearly independent from the input into the metabolic chain. A detailed mathematical analysis of such systems has shown, however, that they may

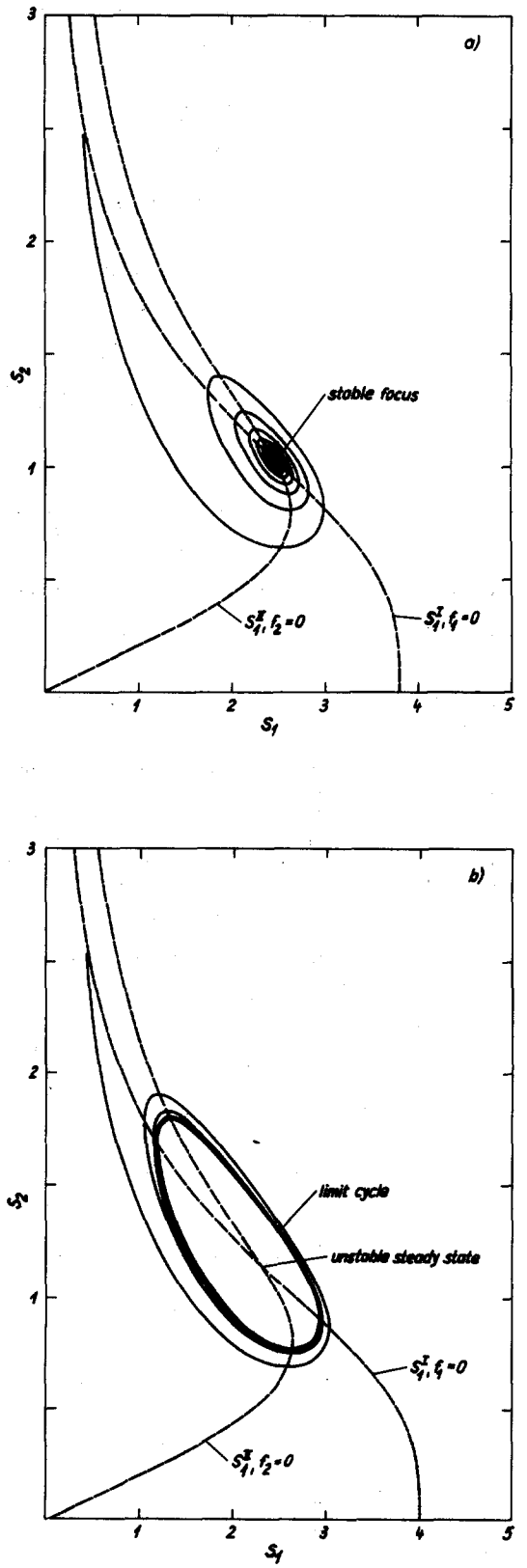



FIG. 6. Trajectories of the reaction system represented in Scheme 3 (a) $v_0 = 7.5$, $c = 1.0$; the steady state is a stable focus. (b) $v_0 = 8.0$, $c = 1.0$; the steady state is an unstable focus and the trajectories approach a limit cycle. The other parameter values are the same as given in the legend to Fig. 5. The dotted curves represent the quasi-steady state lines defined by eqns. (3.26a,b).

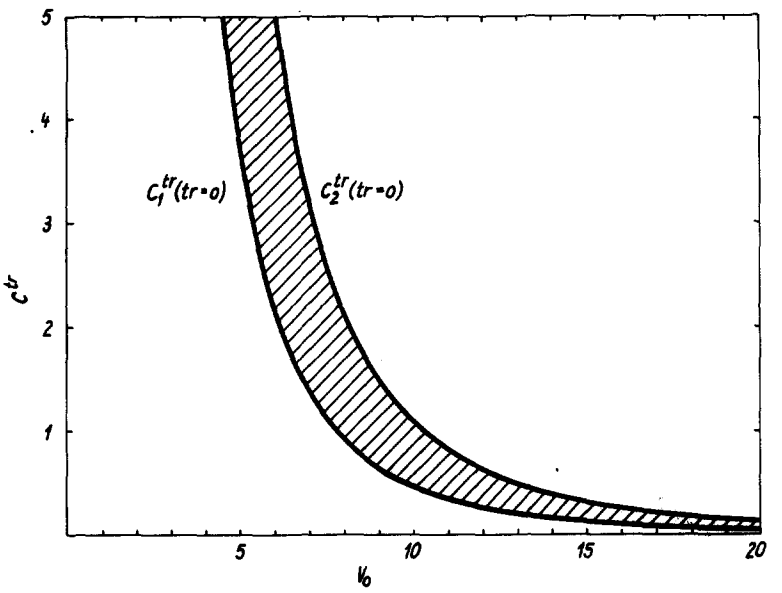



FIG. 7. Parameter regions with different numbers of steady states for the reaction system represented in Scheme 3. Parameter values: $k_1 = 1$, $k_{-1} = 0$, $k_2 = 5$, $k_3 = 5$, $q = 3$. Within the hatched area the system exhibits three steady states for each set of the parameters $c$ and $v_0$. In the other parameter region the steady state is unique.

exhibit a periodic behaviour of the concentrations, if a critical degree of inhibition is exceeded. The steady states of such systems become dynamically unstable (Morales and McKay, 1967; Viniegra and Martinez, 1969; Walter, 1970; Higgins, 1973; Hunding, 1974; Savageau, 1975; Tyson, 1975). The tendency towards instability grows with increasing size of the system. In the following section it is shown that the occurrence of oscil-

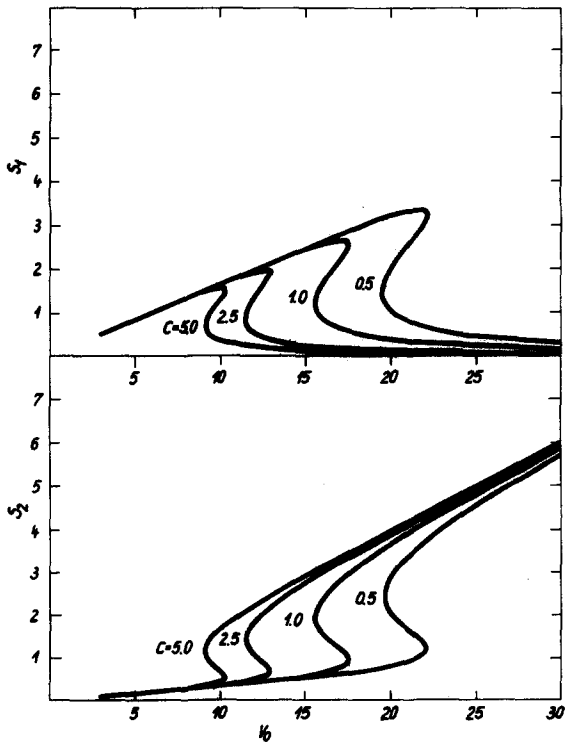



FIG. 8. Occurrence of multiple steady states in the reaction system represented in Scheme 3. The parameter values are the same as given in the legend to Fig. 7. $v_0$ and $c$ are varied.

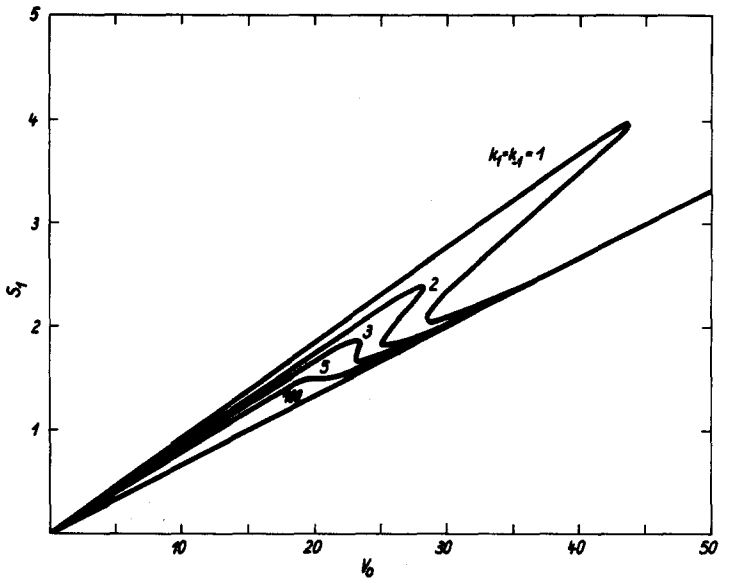
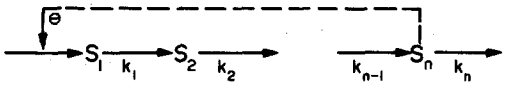



FIG. 9. Disappearance of the multiplicity of steady states by acceleration of the back activated reaction of Scheme 3. $k_1$ and $k_{-1}$ are varied while the equilibrium constant $k_1/k_{-1}$ remains constant. The other parameters are $k_2 = 5$, $k_3 = 10$, $c = 1$, $q = 6$.

lations in linear systems with feedback-inhibition may be explained within the framework of the theory of bifurcations.

The system considered is depicted in Scheme 4.




Scheme 4.

For the sake of simplicity it is presupposed that all enzymes catalyze first order reactions, with the exception of the first enzyme, which is inhibited by $S_n$. For its activity the following relation is assumed

$$v_0 = \frac{v}{1 + pS_n^q}. \tag{3.37}$$

One obtains the differential equations

$$\frac{dS_1}{dt} = \frac{v}{1 + pS_n^q} - k_1 S_1 \tag{3.38a}$$

$$\frac{dS_i}{dt} = k_{i-1} S_{i-1} - k_i S_i \qquad (i = 2, \ldots, n). \tag{3.38b}$$

The expressions for the steady state concentrations are

$$S_i^0 = \frac{k_{i-1}}{k_i} S_i^0 \qquad (i = 2, \ldots, n) \tag{3.39a}$$

and

$$v_0 - k_1 S_1^0 - p\left(\frac{k_1}{k_n}\right)^q k_1 (S_1^0)^{q+1} = 0. \tag{3.39b}$$

By means of a transformation of the variables (Hunding, 1974) the nonlinear Eqn. (3.39b) may be written in the manner

$$c\sqrt[q]{c-1} = \frac{v}{k_n}\sqrt[q]{p}. \tag{3.40}$$

From it $c$, and therefore $S_1^0$ may be calculated by numerical methods. For the analysis of the stability behaviour of the system solutions of the differential equations (3.38a,b) are investigated in a region close to the steady state.

One obtains the following characteristic polynomial

$$\prod_{i=1}^{n}(k_i + \lambda) + \frac{c-1}{c} q \prod_{i=1}^{n} k_i = 0. \tag{3.41}$$

Simple solutions can only be found if all $k_i$ are equal ($k_i = k$). In this case one obtains for the eigenvalues

$$\lambda_m = -k + k \exp\left[i\left(\frac{\pi}{n} + \frac{2\pi}{n} m\right)\right] \sqrt[n]{q \frac{c-1}{c}}. \tag{3.42}$$

They lie in the plane of complex numbers on a circle with the centre $(-k)$ which is located on the negative real axis and has the radius

$$r = k \sqrt[n]{q \frac{c-1}{c}}. \tag{3.43}$$

From Eqn. (3.42) it may be seen that the stability of the system depend critically on the location of the eigenvalue $\lambda_0$. Thus, one obtains from Eqn. (3.42) the following condition for the emergence of an unstable steady state and a limit cycle

$$q > \left(\frac{c}{c-1}\right) \frac{1}{\cos^n(\pi/n)} = q_0. \tag{3.44}$$

Given a chain length of $n$ metabolites limit cycles occur only if the feedback inhibition is sufficiently strong. For $c \gg 1$ (this is the case if one of the parameters on the right side of Eqn. (3.40) is very large), the expression $c/(c-1)$ tends to one and the condition (3.44) for the instability becomes independent of the steady state concentrations of the metabolites

$$q > \frac{1}{\cos^n(\pi/n)} = q_0'. \tag{3.45}$$

This simplified condition (3.45) has been first derived by Viniegra and Martinez (1969). Table 2 shows the critical inhibition parameter $q_0'$ for different chain lengths $n$. It may be seen that $q_0'$ becomes smaller as $n$ increases. Thus the tendency for a limit cycle to occur increases with greater feedback inhibition as well as with growing chain length.

TABLE 2. CRITICAL FEEDBACK-INHIBITION PARAMETER $q_0'$ DEFINED BY FORMULA (3.45) AS A FUNCTION OF THE CHAIN LENGTH $n$ OF AN UNBRANCHED METABOLIC PATHWAY

| $n$ | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|
| $q_0'$ | 8.0 | 4.0 | 2.9 | 2.4 | 2.1 | 1.9 | 1.7 | 1.6 |

If the kinetic constants $k_i$ differ from each other the stability has to be checked by means of the Routh–Hurwitz-criterion. We shall limit ourselves to the case $n = 3$. The characteristic equation is then, from Eqn. (3.41),

$$\lambda^3 + \lambda^2(k_1 + k_2 + k_3) + \lambda(k_1k_3 + k_2k_3 + k_1k_2) + k_1k_2k_3\left(1 + q\frac{c-1}{c}\right) = 0. \tag{3.46}$$

According to the Routh–Hurwitz-criterion the system becomes unstable if

$$(k_1 + k_2 + k_3)(k_1k_3 + k_2k_3 + k_1k_2) > k_1k_2k_3\left(1 + q\frac{c-1}{c}\right) \tag{3.47a}$$

or

$$q > \frac{c}{c-1}\left(2 + \frac{k_1}{k_2} + \frac{k_2}{k_1} + \frac{k_2}{k_3} + \frac{k_3}{k_2} + \frac{k_1}{k_3} + \frac{k_3}{k_1}\right) = q_0. \tag{3.47b}$$

Since $c$ is independent of $k_1$ and $k_2$ (see Eqn. (3.40)), the right side of the inequality (3.47b) becomes minimal if all $k_i$ are equal. The stability region of the system increases therefore if the rate constants differ from each other. This has been shown for systems $n > 3$ by Savageau (1975).

One may assume that during evolution the regulatory mechanism based on end-product inhibition has been instrumental in the direction of increased stability of steady states rather than of the production of limit cycles. The question arises in which manner feedback systems are protected against dynamic instabilities. The results presented indicate two mechanisms for the stabilization (see also Savageau, 1975).

(1) Minimization of the length of unbranched pathways with feedback inhibition.
(2) Optimization of the kinetic parameters in such a way that great differences in their magnitudes are achieved.

The first possibility seems to be of lesser importance since the number of reactions in a metabolic pathway are essentially determined by the chemical differences between the first substrate and the end-product. There are some indications that the second possibility, i.e. the differentiation of kinetic parameters has been realized in nature. The histidin biosynthetic pathway of the bacterium *Salmonella typhimurium* consists of an unbranched chain of ten reactions. The system would be probably unstable, if all the reaction constants were of the same magnitude (see Table 2). Since the kinetic constants and therefore, the steady state concentrations of the metabolites differ about sixtyfold (Savageau, 1975) the occurrence of unstable steady states is practically eliminated. The fact that systems are more stable if they involve widely different kinetic constants will be discussed in a more general context in Section III.5.(c).

### 5. *Time Hierarchy in Metabolic Systems*

It has been emphasized before that there exists a *time hierarchy* in biological systems. It results from the fact that the systems involve simultaneously many reactions which are not independent and which take place with different velocities. At first we shall consider the methodological consequences of this phenomenon for the modelling of complex metabolic systems and shall discuss afterwards its biological relevance.

As a result of the time hierarchy the motion of a metabolic system may be differentiated into a sequence of *epochs* characterized by the time constants for the relaxation of the variables. Both from experimental and theoretical reasons an epoch of a certain time range is selected for the study. Some variables are so rapid that it may be assumed that they are already relaxed, i.e. that they are in a quasi-steady state. On the other hand, other variables are so slow that they remain approximately constant during the epoch considered. Therefore, one is left with a few essential variables which have to be described by differential equations.

#### (a) *Relaxation Times*

A mathematical description of the time hierarchy of systems requires a quantitative measure. One is provided by the eigenvalues of the dynamic system considered (see Section III.2). Their reciprocals are the *relaxation times* which indicate within which time the motions return to their steady state after perturbation. The system contains as many eigenvalues as variables. If it is hierarchically structured in time, its eigenvalues are widely different. It should be emphasized once more, that the eigenvalues characterize the system only in a close neighbourhood of the steady state where the linear approximation is applicable. The possibility to define relaxation times in non-linear systems will be discussed in Section IV.4.

The relaxation of an isolated reaction may be described by only one time constant. For a monomolecular reaction $S_1 \rightleftarrows S_2$, the dynamics of which is described by

$$\frac{dS_1}{dt} = -v_1(S_1) + v_{-1}(S_2), S_1 + S_2 = \text{const.}, \tag{3.48}$$

one obtains in the neighbourhood of the equilibrium the following differential equation

$$\frac{d\sigma_1}{dt} = -\left(\frac{dv_1}{dS_1} + \frac{dv_{-1}}{dS_2}\right)\sigma_1. \tag{3.49}$$

The solution of this equation is

$$\sigma_1(t) = \sigma_1(t_0)\exp\left[-\frac{(t-t_0)}{\tau}\right] \tag{3.50}$$

with the relaxation time

$$\tau = \frac{1}{\dfrac{dv_1}{dS_1} + \dfrac{dv_{-1}}{dS_2}}. \tag{3.51a}$$

If the reaction may be described by first order rate constants ($v_1 = k_1 S_1$, $v_{-1} = k_{-1} S_2$) one obtains for the relaxation time

$$\tau = \frac{1}{k_1 + k_{-1}}. \tag{3.51b}$$

Analogously, one obtains for the bimolecular reaction $S_1 + S_2 \rightleftarrows S_3 + S_4$ for the relaxation time

$$\tau = \frac{1}{k_1(S_1^0 + S_2^0) + k_{-1}(S_3^0 + S_4^0)} \tag{3.52}$$

in which $S_i^0$ represents the values of the metabolites at equilibrium.

(b) *Steady State Approximation*

Differential equations with widely different time constants are "stiff". The overall motion of the system proceeds with a velocity which is characterized by the relaxation times of the slow processes even though the system contains rapid motions. In the usual numerical methods of integration the step width $\tau_s$ is determined by the values of the smallest relaxation time of the system

$$\tau_s \sim \min\left(-\frac{1}{\lambda i}\right). \tag{3.53}$$

Therefore the number of iterations required is very high and the integration procedure becomes rather cumbersome. It is one of the tasks of the theorists to find economical methods which permit one to find acceptable approximate solutions.

We shall suppose that the time constants of a system are grouped in such a manner that there exist two widely separated epochs. Then, the state vector **S** may be subdivided into the two vectors **x** and **y**

$$\mathbf{S} = (\mathbf{x}, \mathbf{y}) \tag{3.54}$$

where **x** represents the vector of the slow variables $(x_1, \ldots, x_{n_x})$ the number of which is $n_x$ and **y** the vector of the fast variables $(y_1, \ldots, y_{n_y})$ the number of which is $n_y$. In such cases the differential equations (3.5) may be written frequently in the following manner (Tichonov, 1948)

$$\frac{d\mathbf{x}}{dt} = \mathbf{g}(\mathbf{x}, \mathbf{y}) \tag{3.55}$$

$$\mu\frac{d\mathbf{y}}{dt} = \mathbf{G}(\mathbf{x}, \mathbf{y}) \tag{3.56}$$

where $\mu$ represents a very small parameter ($\mu \ll 1$). **g** denotes the vector $(g_1, \ldots, g_{n_x})$

of the net flux functions of the slow variables and **G** the vector $(G_1,\ldots,G_{n_y})$ of the net flux functions of the fast variables. The factor $\mu$ in the original differential equations may be split off for two reasons:

(1) because the concentrations of some metabolites are much lower than those of some others. One obtains the factor $\mu$ by normalizing all variables to one concentration scale;
(2) because the fluxes among metabolites differ widely in their magnitudes. In that case the factor $\mu$ is obtained by normalization of the fluxes to one time scale.

It should be emphasized that the splitting off is generally only permissible for certain domains of the state space, where both **x** and **y** as well as **g** and **G** are of the same order of magnitude.

To apply the *steady state approximation* for the fast variables **y** means that we take in Eqns. (3.55) and (3.56) the limit transition $\mu \to 0$. Thus we obtain the dynamical system

$$\frac{\mathrm{d}\mathbf{x}}{\mathrm{d}t} = \mathbf{g}(\mathbf{x}, \mathbf{y}) \tag{3.57a}$$

$$0 = \mathbf{G}(\mathbf{x}, \mathbf{y}). \tag{3.57b}$$

In this equation system the number of the dynamic variables is reduced by the number of fast variables. Equation (3.57b) defines in the state space a surface **G** which contains the quasi-steady states of the system. The differential equation (3.57a) determine the slow motion in this surface. The question to be answered is, under which conditions the simplified system (3.57) describes satisfactorily the motion of a system determined by the complete Eqns. (3.55) and (3.56).

We consider firstly the expression

$$\left|\frac{\mathrm{d}y_i}{\mathrm{d}x_j}\right| = \frac{1}{\mu}\left|\frac{G_i(x_j, y_i)}{g_j(x_j, y_i)}\right| \tag{3.58}$$

which specifies the location of the trajectories of the complete system. Outside the close neighbourhood of the surface **G**, i.e. for $(x, y)$-values where the numerator in Eqn. (3.58) is not equal to zero one gets in the limiting case $\mu \to 0$

$$\left|\frac{\mathrm{d}y_i}{\mathrm{d}x_j}\right| \to \infty\,. \tag{3.59}$$

This expression means that outside of the surface **G** very large changes of **y** are accompanied by only very small changes of **x**. The trajectories lie in this region of the state space approximately along

$$x_i = x_i^0 = \text{const.} \tag{3.60}$$

The motion of the system on these lines is very fast since it is determined by the differential equations of the fast variables alone (Eqn. (3.56)). The trajectories are directed towards the surface **G** if the rapid subsystem (3.56) is stable, i.e. if the submatrix

$$A'_{ik} = \frac{\partial G_i}{\partial y_k} \tag{3.61}$$

of the whole Jacobi-matrix $A_{ik}$ has only eigenvalues with negative real parts. Otherwise, the system moves away from the surface **G**. Stable states of the rapid subsystem are called *attractors* and unstable states *repellors*. The stability character of the rapid sub-system must be distinguished from the stability character of the whole system which is additionally determined by the slow subsystem. If the surface **G** is an attractor, the system arrives in the close neighbourhood within a very short time period. The duration

of the initial rapid motion decreases with smaller values of $\mu$. A detailed analysis shows that the fast variables relax within a time $\Delta t$ which is approximately given by (Vasilieva and Butuzov, 1973)

$$\Delta t \sim \mu\left(\ln\frac{1}{\mu}\right). \tag{3.62}$$

After the relaxation the subsequent motion occurs only in the close neighbourhood of the surface **G**. One may conclude that for times $t > t_0 + \Delta t$ the motion of the system defined by Eqns. (3.55) and (3.56) is satisfactorily described by the simplified equation system (3.57). The situation is shown in Fig. 10 for the three-dimensional case with two slow and one fast variable, ($\mathbf{S} = (x_1, x_2, y_1)$).

Equation system (3.57) which is obtained by the steady state approximation may be solved in the following way. The solution $\mathbf{y}(x)$ of Eqn. (3.57b) is inserted into Eqn. (3.57a) so that the latter becomes a function of the slow variables **x** alone, and can be integrated. However, Eqn. (3.57b) can only be easily solved if **G** is a linear function of **y**. Generally, however, the implicit Eqn. (3.57b) for the quasi-steady states are non-linear functions of the variables, so that their explicit representation in an analytical manner is no longer possible. In such a case the coupled system of Eqns. (3.57a,b) must be solved by numerical iterative procedures. This may be done in the following way (e.g. Park, 1974): At a time $t$ after an iterative solution of the non-linear Eqn. (3.57b) at fixed values of the slow variable **x**, one performs an integrative step of the system (3.57a) which leads to new values of the slow variable at a time $t + \Delta t$. These are again used to derive new values of $\mathbf{y}(t + \Delta t)$ by means of Eqn. (3.57b). This procedure ensures that at any time the initial values of **y** lie near the sought steady state values so that the number of iterations always remains small.

The methods so far described are based on a preliminary classification of the fluxes and the metabolite concentrations before the integration is performed. Park (1974) has proposed another method which permits the classification of the variables by means of a computer. One starts with a numerical integration of the complete system of differential equations and tests at every step whether the steady state approximation is applicable to any of the variables. The value of

$$\epsilon_i = \left|\frac{f_i^+ - f_i^-}{f_i^-}\right| = \left|\frac{f_i}{f_i^-}\right| \tag{3.63}$$

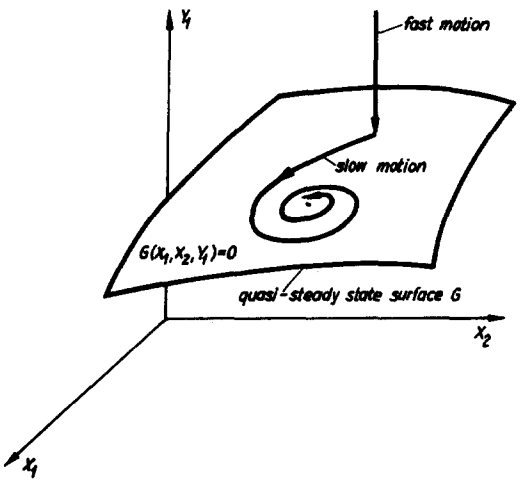



FIG. 10. Quasi-steady state surface and trajectory in a three-dimensional state space. In the Scheme one fast variable $y$ and two slow variables $x_1$ and $x_2$ are assumed. Equation (3.57b) defines a two-dimensional surface **G**. During the motion two epochs can be distinguished. At first, the surface **G** is approached by rapid changes of the fast variable $y$. After that the system moves slowly within this plane towards the steady state.

is used as criterion, i.e. one compares for each variable the ratio between the net flux and the out-flux. All metabolites for which $\epsilon_i$ is smaller than a preset limit, form the set of the rapid variables and are considered to be in a steady state.

Frequently it will not be possible to transform directly the original differential equations (3.5) to a system of the structure of Eqns. (3.55) and (3.56) since the metabolites may be influenced simultaneously by rapid and slow fluxes. Therefore, one obtains in the general case after normalization of the fluxes the expression

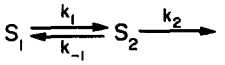
$$\frac{dS_i}{dt} = \sum_{j=1}^{r} c_{ij}^1 v_j + \frac{1}{\mu} \sum_{k=1}^{r} c_{ik}^2 v_k. \tag{3.64}$$

The stoichiometric matrix is divided into two components, one of which, $c_{ij}^1$, represents the influence of the slow fluxes, while the other, $c_{ik}^2$, that of the rapid fluxes on $S_i$. To transform systems of the structure of Eqn. (3.64) into the form of Eqns. (3.55) and (3.56) a mathematical transformation of the variables is necessary. Because of the special structure of the right side of Eqn. (3.64) the slow variables may be represented as linear combinations of the original variables. In this way one obtains the compound pools

$$x_i = \sum_{j=1}^{n} w_{ij} S_j \qquad (i = 1, \ldots, n_x) \tag{3.65}$$

the so-called *slow moieties*, the changes of which are determined only by slow fluxes. $w_{ij}$ are the elements of a matrix which determines the transformation of the variables.

(i) *Application to a Two-Enzyme System with Widely Different Reaction Rates.* In this example we consider a system with two components which is depicted in Scheme 5.




Scheme 5.

The dynamics of this system is described in a linear approximation by the following equations

$$\frac{dS_1}{dt} = -k_1 S_1 + k_{-1} S_2 \tag{3.66a}$$

$$\frac{dS_2}{dt} = k_1 S_1 - k_{-1} S_2 - k_2 S_2. \tag{3.66b}$$

It is presupposed that the first enzyme catalyzes a very fast reaction and the second a very slow one. We shall define the small parameter $\mu$ in the following manner

$$\mu = \frac{k_2}{k_{-1}} = \frac{k_1}{k_{-1}} \frac{k_2}{k_1} = q_1 \frac{k_2}{k_1}. \tag{3.67a}$$

After normalization of the time scale

$$t = k_2 t, \tag{3.67b}$$

one obtains

$$\frac{dS_1}{dt^*} = \frac{1}{\mu}(-q_1 S_1 + S_2) \tag{3.68a}$$

$$\frac{dS_2}{dt^*} = -\frac{1}{\mu}(-q_1 S_2 + S_2) - S_2. \tag{3.68b}$$

As expected both equations contain $\mu$. It is easily seen that the system contains the slow moiety $S_1 + S_2$. If one sets $S_1 + S_2 = x$ and $S_2 = y$ the following expressions are obtained

$$\frac{d\mathbf{x}}{dt^*} = \mathbf{y} - \mathbf{x} = \mathbf{g}(\mathbf{x}, \mathbf{y}) \tag{3.69a}$$

$$\mu \frac{d\mathbf{y}}{dt^*} = -(q_1 + 1)\mathbf{y} + \mathbf{x} = \mathbf{G}(\mathbf{x}, \mathbf{y}). \tag{3.69b}$$

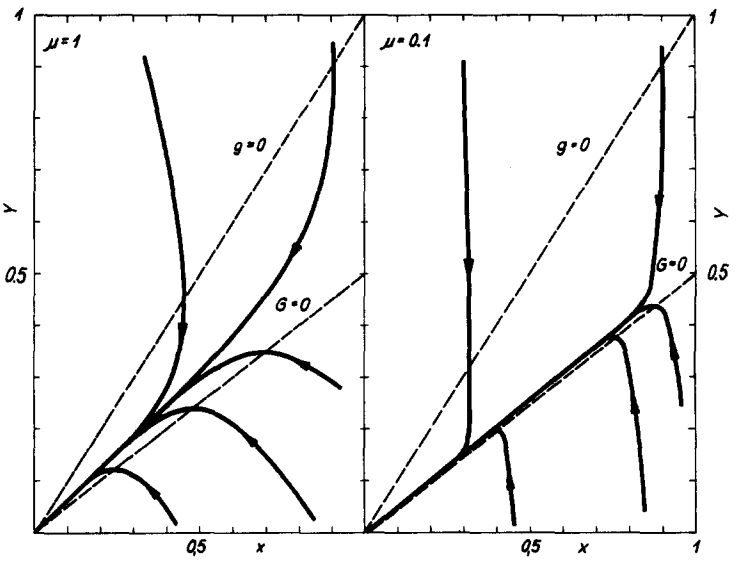



FIG. 11. Trajectories and quasi-steady state lines in the $(x,y)$-phase plane of a linear two-component system (reaction Scheme 5). The reversible reaction is slow in the left side ($\mu = 1$) and fast in the right side ($\mu = 0.1$). For the equilibrium constant the value $q_1 = 1$ is used.

Figure 11 shows the trajectories for different values of $\mu$, which were obtained by numerical integration of the equation system (3.69).

Depending on whether the rapid or the slow process is considered, one may choose an appropriate approximation procedure.

(a) Consideration of the rapid process:

The approximation presupposed is

$$\frac{dx}{dt^*} = 0, \quad S_1 + S_2 = x_0 = \text{const.}$$

For the Eqn. (3.69b) one obtains in the system of the coordinates of the original variables

$$\frac{dS_1}{dt} = -(k_1 + k_{-1})S_1 + x_0. \tag{3.70}$$

The initial movement occurs therefore with the very short relaxation time $\tau = 1/(k_1 + k_{-1})$.

(b) Consideration of the slow process:

Here the approximation presupposed is $G = x - (q_1 + 1)y = 0$. This approximation is equivalent with $S_2 = q_1 S_1$, i.e. with the assumption of equilibrium for the partners of the rapid reaction. One obtains

$$\frac{dx}{dt^*} = \frac{d(S_1 + S_2)}{dt} = \frac{(1 + q_1)}{k_2}\frac{dS_1}{dt} = -q_1 S_1. \tag{3.71}$$

The solution of this equation describes a relaxation motion with the characteristic time

$$\tau_2 = \frac{1}{k_2}\left(\frac{1 + q_1}{q_1}\right).$$

By means of Eqn. (3.67a) one obtains a relation between $\tau_1$ and $\tau_2$,

$$\tau_1 = \mu \frac{q_1}{(1 + q_1)^2}\tau_2. \tag{3.72}$$

Since $\mu$ is very small and the fractional factor on the right side of Eqn. (3.72) never exceeds the value of 1/4, $\tau_1$ is always much smaller than $\tau_2$.

(ii) *Application to an Isolated Enzyme Reaction with Small Enzyme Concentration.* In the following we consider the reactions of an isolated enzyme and take into account not only the variations of the metabolite concentrations but also those of the intermediate complexes between the enzyme and the metabolites. It will be shown that for the case that the enzyme concentrations are very small compared with the metabolite concentrations the steady state approximation may be applied for the intermediate complexes. The example shows for a special enzyme mechanism under which circumstances it is justified to describe metabolic systems by steady state equations which contain only the metabolites as variables.

Scheme 6 shows the enzyme catalyzed transformation of two substrates $S_1$ and $S_2$.

$$S_1 + E \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} E^* \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} S_2 + E$$

Scheme 6.

$E$ indicates the free enzyme and $E^*$ the intermediate complex. For this mechanism the following differential equations hold

$$\frac{dS_1}{dt} = -k_1 S_1 E + k_1 E^* \tag{3.73a}$$

$$\frac{dS_2}{dt} = -k_{-2} S_2 E + k_2 E^* \tag{3.73b}$$

$$\frac{dE^*}{dt} = k_1 S_1 E - (k_{-1} + k_2) E^* + k_{-2} S_2 E \tag{3.73c}$$

$$\frac{dE}{dt} = -k_1 S_1 E + (k_{-1} + k_2) E^* - k_{-2} S_2 E. \tag{3.73d}$$

The system contains two conservation quantities, the total metabolite concentration $S_0$ and the total enzyme concentration $E_0$

$$S_1 + S_2 + E = S_0, E + E^* = E_0. \tag{3.74}$$

In many biochemical systems the concentration of the enzymes are much smaller than those of the substrates. One may therefore assume that

$$\mu = \frac{E_0}{S_0} \ll 1. \tag{3.75}$$

After normalization of the variables to the same scale

$$x_1 = \frac{S_1}{S_0}, \quad x_2 = \frac{S_2}{S_0}, \quad y_1 = \frac{E^*}{E_0} \tag{3.76}$$

and introduction of dimensionless kinetic parameters

$$\mathscr{K}_1 = k_1 E_0, \mathscr{K}_{-1} = k_{-1} \frac{E_0}{S_0}, \ \mathscr{K}_2 = k_2 \frac{E_0}{S_0}, \ \mathscr{K}_{-2} = k_{-2} E_0 \tag{3.77}$$

one obtains after elimination of $E$ from Eqn. (3.73) the following expressions

$$\frac{dx_1}{dt} = \mathscr{K}_1 x_1 y_1 - \mathscr{K}_1 x_1 + \mathscr{K}_{-1} y_1 = g_1 \tag{3.78a}$$

$$\frac{dx_2}{dt} = \mathscr{K}_{-2} x_2 y_1 - \mathscr{K}_{-2} x_2 + \mathscr{K}_2 y_1 = g_2 \tag{3.78b}$$

$$\mu \frac{dy_1}{dt} = \mathscr{K}_1 x_1 - (\mathscr{K}_{-1} + \mathscr{K}_2 + \mathscr{K}_1 x_1 + \mathscr{K}_{-2} x_2) y_1 + \mathscr{K}_{-2} x_2 = G_1. \tag{3.78c}$$

Since $\mu$ is very small one may take the metabolite concentrations $x_1$ and $x_2$ to be constant for very short times. In that case Eqn. (3.78c) is a linear differential equation for the normalized concentration of the intermediate $y_1$. The solution is an exponential function, which describes the transition of $E^*$ to the steady state. This process has the relaxation time

$$\tau = (k_{-1} + k_2 + k_1 S_1 + k_{-2} S_2)^{-1}. \tag{3.79}$$

For periods $t \gg \tau$ the steady state approximation for $y_1$ is applicable ($\mu \to 0$) and one obtains

$$y_1^0 = \frac{\mathscr{H}_1 x_1 + \mathscr{H}_{-2} x_2}{\mathscr{H}_{-1} + \mathscr{H}_2 + \mathscr{H}_1 x_1 + \mathscr{H}_{-2} x_2}. \tag{3.80}$$

By insertion of Eqn. (3.80) into Eqns. (3.78a,b) and reverse transformation into the original variables one arrives at the differential equation

$$\frac{dS_1}{dt} = -\frac{dS_2}{dt} = -\frac{E_0(k_1 k_2 S_1 - k_{-1} k_{-2} S_2}{k_{-1} + k_2 + k_1 S_1 + k_{-2} S_2}. \tag{3.81}$$

This equation is the usual Michaelis-relation which depends only on the variables $S_1$ and $S_2$. The total enzyme concentration enters as a parameter. Equation (3.81) may be also written in the following form

$$v = \frac{dS_2}{dt} = \frac{(V_m^1/K_m^1)S_1 - (V_m^{-1}/K_m^{-1})S_2}{1 + (S_1/K_m^1) + (S_2/K_m^{-1})}. \tag{3.82}$$

$V_m^1$ and $V_m^{-1}$ denote the maximal velocities of the forward and backward reaction, respectively

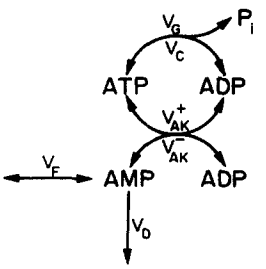
$$V_m^1 = k_2 E_0, \quad V_m^{-1} = k_{-1} E_0 \tag{3.83}$$

and $K_m^1$ and $K_m^{-1}$ defined by

$$k_m^1 = \frac{k_{-1} + k_2}{k_1}, \quad K_m^{-1} = \frac{k_{-1} + k_2}{k_{-2}} \tag{3.84}$$

are the Michaelis-constants of the substrate and product respectively.

(iii) *The Energy Charge as an Essential Variable of Energy Metabolism.* Scheme 7 shows a reaction system which plays a central role in the energy metabolism (see Section 5). It contains the adenylate kinase reaction ($v_{AK}^+$, $v_{AK}^-$), ATP-consuming and generating processes ($v_C$ and $v_G$ respectively) and reactions forming and degrading AMP ($v_F$ and $v_D$, respectively).




Scheme 7.

The dynamics of the system is described by the following differential equations

$$\frac{\mathrm{d}[\mathrm{ATP}]}{\mathrm{d}t} = v_{\mathrm{G}} - v_{\mathrm{C}} - v_{\mathrm{AK}}^{+} + v_{\mathrm{AK}}^{-} \tag{3.85a}$$

$$\frac{\mathrm{d}[\mathrm{ADP}]}{\mathrm{d}t} = -v_{\mathrm{G}} + v_{\mathrm{C}} + 2v_{\mathrm{AK}}^{+} - 2v_{\mathrm{AK}}^{-} \tag{3.85b}$$

$$\frac{\mathrm{d}[\mathrm{AMP}]}{\mathrm{d}t} = v_{\mathrm{F}} - v_{\mathrm{D}} - v_{\mathrm{AK}}^{+} + v_{\mathrm{AK}}^{-}. \tag{3.85c}$$

Usually, the fluxes which enter Eqns. (3.85a–c) are of different orders of magnitudes. The processes forming and degrading AMP are much slower and the adenylate kinase reaction is much faster than the processes which consume and generate ATP. After normalization of the fluxes to the same scale (cf. Reich and Selkov, 1974) Eqns. (3.85a–c) may be transformed into the system

$$\frac{\mathrm{d}([\mathrm{AMP}] + [\mathrm{ADP}] + (\mathrm{ATP}])}{\mathrm{d}t} = \mu_2(\tilde{v}_{\mathrm{F}} - \tilde{v}_{\mathrm{D}}) \tag{3.86a}$$

$$\frac{\mathrm{d}([\mathrm{ATP}] + \frac{1}{2}[\mathrm{ADP}])}{\mathrm{d}t} = \tfrac{1}{2}(v_{\mathrm{G}} - v_{\mathrm{C}}) \tag{3.86b}$$

$$\mu_1 \frac{\mathrm{d}[\mathrm{AMP}]}{\mathrm{d}t} = \mu_1\mu_2(\tilde{v}_{\mathrm{F}} - \tilde{v}_{\mathrm{D}}) - \tilde{v}_{\mathrm{AK}}^{+} + \tilde{v}_{\mathrm{AK}}^{-}. \tag{3.86c}$$

$\tilde{v}_{\mathrm{AK}}^{+}$, $\tilde{v}_{\mathrm{AK}}^{-}$, $\tilde{v}_{\mathrm{F}}$ and $\tilde{v}_{\mathrm{D}}$ denote the normalized fluxes which are of the same order as $v_{\mathrm{C}}$ and $v_{\mathrm{G}}$. $\mu_1$ and $\mu_2$ are small parameters arising from the different magnitudes of the fluxes. It is seen that for the motion described by Eqns. (3.86a–c) three time scales may be distinguished. The first is determined by the rapid change of AMP, the second by the motion of the pool (ATP + 1/2ADP) and the third by the slow changes of the total sum $A$ of the adenine nucleotides. The limit transition $\mu_1 \to 0$, $\mu_2 \to 0$ gives

$$[\mathrm{AMP}] + [\mathrm{ADP}] + [\mathrm{ATP}] = A = \text{const.} \tag{3.87}$$

and

$$\tilde{v}_{\mathrm{AK}}^{+} - \tilde{v}_{\mathrm{AK}}^{-} = 0; \text{ i.e. } k_{\mathrm{AK}}^{+}[\mathrm{AMP}][\mathrm{ATP}] = k_{\mathrm{AK}}^{-}[\mathrm{ADP}]^2. \tag{3.88}$$

Equation (3.87) shows that the total sum of the adenine nucleotides is conserved during the intermediate time period. Equation (3.88) is identical with the equilibrium condition for the adenylate kinase reaction. In this approximation the dynamics of the system is described by only one essential variable. Owing to the constancy of $A$ one can use as the essential variable

$$x = \frac{[\mathrm{ATP}] + \frac{1}{2}[\mathrm{ADP}]}{[\mathrm{AMP}] + [\mathrm{ADP}] + [\mathrm{ATP}]}, \tag{3.89}$$

which is identical to the *energy charge* defined by Atkinson (1968) and Atkinson *et al.* (1975). The numerator contains the metabolically available phosphoryl groups in the adenylate pool (2 ADP are equivalent to 1 ATP owing to the adenylate kinase reaction).

(iv) *Estimate of the Error in the Steady State Approximation.* The procedure outlined to deal with systems of differential equations which describe coupled motions with greatly different velocities is only exact if the time constants of the rapid motions are infinitesimally small. Therefore, an error is always introduced if the steady state approximation is applied to any real system. After an initial relaxation the system moves in a certain distance of the surface **G** which is determined by the steady state condition for the fast variables. The principle of a method to estimate the error incurred is illustrated for the two-dimensional case. In Fig. 12 is shown the $(x, y)$-phase plane which

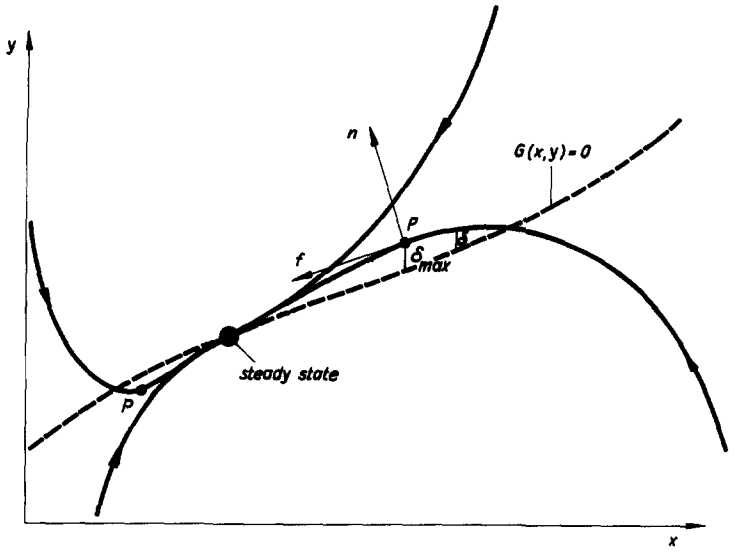



FIG. 12. Definition of the maximal deviation $\delta_{max}$ of the trajectories from the quasi-steady state line $\mathbf{G}(x,y) = 0$ after the initial relaxation phase for a two-component system.

is divided by the quasi-steady state line ($\mathbf{G}(x, y) = 0$). Since we assume that $\mathbf{y}$ is the fast variable, the trajectories shown approach the quasi-steady state line which is assumed to be stable nearly vertically, i.e. parallel to the $y$-axis. For each value of the slow variable $x$ there may be found a trajectory for which the direction of the motion given by the vector $\mathbf{f} = (\mathbf{g}, \mathbf{G})$ is perpendicular to the normal of the quasi-steady state surface $\mathbf{G}$ given by the vector $\mathbf{n} = (\partial\mathbf{G}/\partial x, \partial\mathbf{G}/\partial y)$ (point $P$ in Fig. 12). This means that the scalar product of $\mathbf{f}$ and $\mathbf{n}$ disappears

$$\frac{\partial \mathbf{G}}{\partial x}\mathbf{g} + \frac{\partial \mathbf{G}}{\partial y}\mathbf{G} = 0. \tag{3.90}$$

At the point $P$ the deviation $\delta$ of the trajectory from the quasi-steady state line has a maximum ($\delta = \delta_{max}$). Equation (3.90) can be used to calculate $\delta_{max}$ as a function of the slow variable $x$. Its maximal value in a given interval ($x_a < x < x_b$) is denoted by $\Delta x_a x_b$. Tichonov (1948) has proved that a trajectory which has passed the point $P$ defined by Eqn. (3.90) does not surpass the value $\Delta x_a x_b$ during the further motion in this interval of the slow variable. $\Delta x_a x_b$ is therefore a measure of the maximal deviations of the trajectories from the quasi-steady state lines after the completion of the rapid relaxation. The use of Eqn. (3.90) for the determination of $\delta_{max}$ is difficult owing to its non-linear nature. Park (1974) has proposed a simple procedure for the determination of $\delta_{max}$ which is based on a linearization with respect to small deviations $\delta$. If the system contains several slow variables $x_i$ but only one fast variable $y$, Eqn. (3.90) must be replaced by the equation

$$\sum_i \frac{\partial \mathbf{G}}{\partial x_i} g_i + \frac{\partial \mathbf{G}}{\partial y}\mathbf{G} = 0. \tag{3.91}$$

In systems with several fast variables a corresponding number of $\delta_{max}$-values have to be used for the characterization of the error. As an example we estimate the error involved by the steady state approximation for an isolated enzyme reaction (see Section III.5.(b).(ii)). It is presupposed that the intermediate complex $E$ breaks down irreversibly into the free enzyme $E$ and the product $S_2$. ($k_{-2} = 0$). Furthermore, it is assumed that initially the metabolite is fully in its substrate form ($S_1(t_0) = S_0$). Under these conditions one obtains in the $(S_1, E)$-phase plane for $\delta_{max}$ the following expression

$$\sigma_{max} = (\Delta E^*)_{max} = E^*(S_1)\frac{k_2/k_1 E_0 K_m}{(K_m + S_1)^3}. \tag{3.92}$$

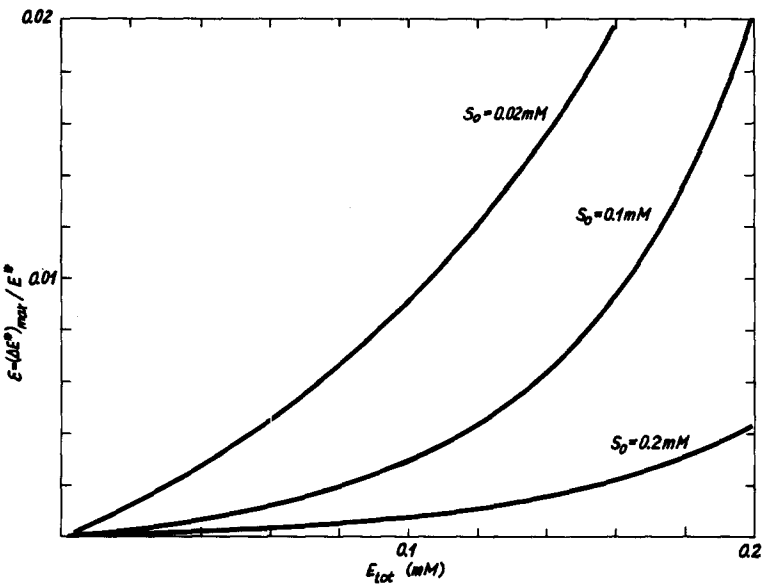



FIG. 13. Relative error $\epsilon = (\Delta E^*)_{max}/E^*$ involved in the steady state approximation for the enzyme substrate intermediate complex for a simple enzyme reaction. Parameter values: $K_m = 0.1$ mM; $k_2/k_1 = 10^{-3}$ mM. The total enzyme concentration and the total substrate concentration are varied.

$S_1$ is the concentration of the substrate after the initial relaxation phase to the quasi-steady state line.

Figure 13 shows the relative error $\epsilon = (\Delta E^*_{max}/E^*)$ as a function of the total enzyme concentration for various total substrate concentrations $S_0$. The kinetic parameters used for the calculation are listed in the legend to the figure. They correspond to realistic values (e.g. Darvey *et al.*, 1975; Dixon and Webb, 1962). As expected the error increases with increasing enzyme concentration $E_0$ and decreasing substrate concentration $S_0$. It is seen that the error increases more than linearly with larger enzyme concentrations. This is explained by the fact that after the initial relaxation phase the substrate is already partly bound to the enzyme or converted to the product. Thus, for a given $S_0$ the substrate concentration $S_1$ is the smaller the higher the enzyme concentration. The relative error remains small even if the enzyme concentration is comparable to the substrate concentration. Equation (3.92) reveals that the fit of the approximation depends also on the ratio of the rate constants $k_2$ and $k_1$. It follows that the steady state approximation is also applicable for high enzyme concentrations if the breakdown of the intermediate $E^*$ into the product is slow compared to the binding of the substrate to the enzyme.

(c) *Metabolic Catastrophes and their Suppression*

As described in the preceding section the motion in a dynamic system with widely differing time constants proceeds in such a manner that after an initial relaxation of the rapid variables only slow changes occur. This conclusion is based essentially on the presupposition that the rapid subsystem has only stable quasi-steady states, and that therefore its motion is confined to the quasi-steady state surface defined by Eqn. (3.57b). We consider now the possibility that this surface is not stable for all values of the slow variables. It is evident, intuitively, that in such a case the rapid variables become essential if the system during its slow motion reaches a region of instability. Then the system may jump into another stable state. Such sudden changes of state are called *catastrophes*. They are the object of a special "theory of catastrophes" which has been developed recently (Thom, 1972).

The investigation of conditions in which such discontinuous changes occur may be of biological importance, since rapid reactions of metabolism which usually are con-

cealed may become of overall importance in certain constellations. It is hardly likely that in the general case the sudden emergence of concealed motions in metabolic systems may be of benefit for their function. Possibly, they occur only if a system is adapted to specialized functions which are connected with rapid motions.

From the theoretical point of view catastrophes have much in common with bifurcations (see Section III.4). While bifurcations are defined by changes of stability behaviour of the *whole* system at variations of the *parameters* of the system, catastrophes occur if the variations of the *slow variables* lead to a loss of stability of a *fast subsystem.* Firstly we shall demonstrate by means of a simple example which kind of dynamic phenomena may be described by the theory of catastrophes. Secondly it will be shown under which conditions the occurrence of catastrophes may be excluded.

(i) *Catastrophes in a Two-Component System.* We consider the two-dimensional system

$$\frac{\mathrm{d}x}{\mathrm{d}t} = y - y_0 = \mathbf{g}(x, y) \tag{3.93a}$$

$$\mu \frac{\mathrm{d}y}{\mathrm{d}t} = y - y^3 - x = \mathbf{G}(x, y) \tag{3.93b}$$

in which $\mu$ is again a small parameter. The quasi-steady state line

$$\mathbf{G}(x, y) = y - y^3 - x = 0 \tag{3.94}$$

is *S*-shaped (Fig. 14a,b). According to the results of Section III.2 the stability characteristics of this line is determined by the expression

$$\frac{\partial \mathbf{G}}{\partial y} = -\frac{\partial \mathbf{G}}{\partial x}\left|\frac{\mathrm{d}y}{\mathrm{d}x}\right. . \tag{3.95}$$

$\mathbf{G} = 0$ is a stable state of the rapid subsystem only if $\partial \mathbf{G}/\partial y < 0$. Since in the present case the numerator in Eqn. (3.95) is always negative only the denominator determines the stability characteristics. It follows immediately that the section $P_1P_2$ of the *S*-shaped line for which $\mathrm{d}y/\mathrm{d}x$ is greater than zero (section $\mathbf{G}^-$) is an repellor. The other two section $\mathbf{G}^+$ are stable and therefore attractors. The positions $x_1$ and $x_2$ of the critical points $P_1$ and $P_2$ are determined by the condition

$$\frac{\partial \mathbf{G}}{\partial y} = 1 - 3y^2 = 0. \tag{3.96}$$

In combination with Eqn. (3.94) one obtains the two solutions

$$x_{1,2} = \pm \tfrac{2}{3}\sqrt{\tfrac{1}{3}}. \tag{3.97}$$

The motion of the system as a whole is determined by the location of the stationary state on the quasi-steady state line. It is defined by the intersection $P$ of the curve $\mathbf{G} = 0$ with the line

$$\mathbf{g} = y - y_0 = 0 \tag{3.98}$$

which runs parallel to the $x$-axis. It is easily demonstrated that the stationary state is stable if the intersection $P$ lies on one of the two stable sections $\mathbf{G}^+$. Figure 14a shows the course of the motion described by the complete differential equations (3.93a,b) from an arbitrarily chosen initial value $A$. Since the parameter $\mu$ has been assumed to be very small ($\mu = 0.02$), the system jumps from the state $A$ to a point $Q_1$ on $\mathbf{G}^+$ and moves thereafter slowly towards the critical point $P_1$, at which again the rapid motion ensues which leads to the point $Q_2$. From it the system moves slowly to the stable stationary state $P$. If the intersection $P$ lies on the unstable section $\mathbf{G}^-$ of the quasi-steady state line (Fig. 14b), there is always an unstable steady state, provided that $\mu$ is sufficiently small. The resulting motion is a limit cycle $C_0{:}Q_1P_1Q_2P_2$, in the

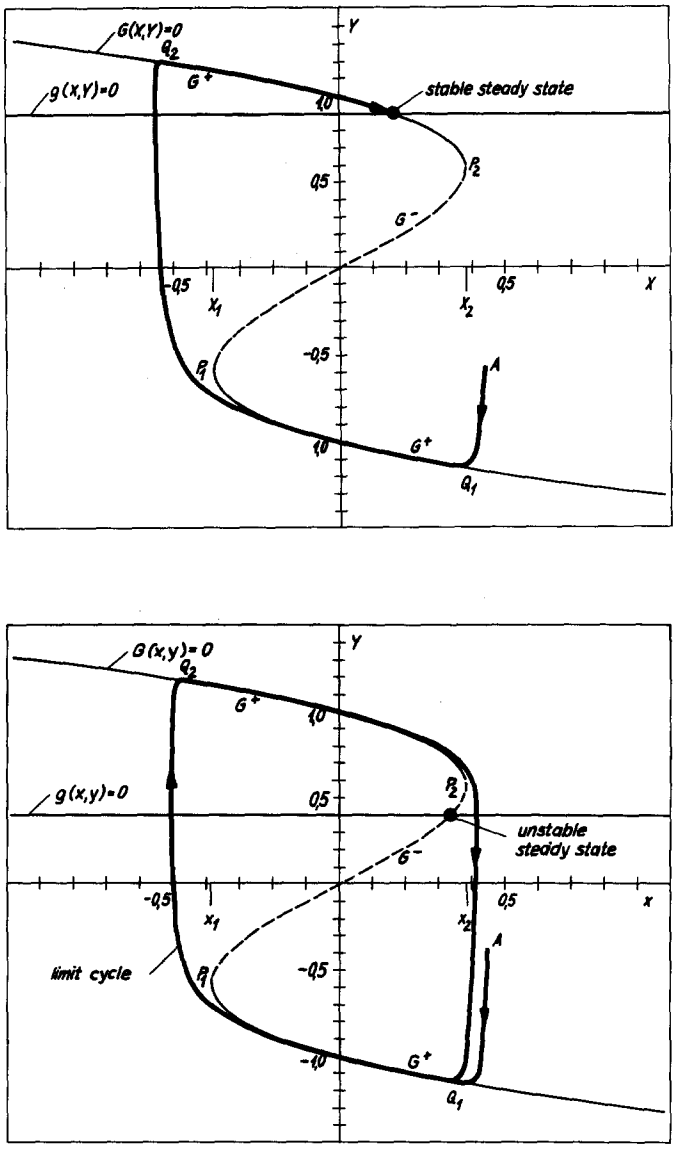



FIG. 14. Quasi-steady state lines $\mathbf{g} = 0$ and $\mathbf{G} = 0$ and trajectories in the $(x, y)$-phase plane of the dynamic system defined by Eqns. (3.93a,b). The dotted lines represent the unstable sections $\mathbf{G}^-$ of the quasi-steady state line $\mathbf{G} = 0$. $P_1$ and $P_2$ are the catastrophic points with the $x$-coordinates $x_1$ and $x_2$, respectively. (a) Relaxation toward a stable steady state. A jump-like transition occurs between the two stable sections $\mathbf{G}^+$ of the quasi-steady state line $\mathbf{G} = 0$. Parameter values: $\mu = 0.02$; $y_0 = 0.9$. (b) Relaxation oscillation around an unstable stationary state. Parameter values: $\mu = 0.02$, $y_0 = 0.4$.

course of which slow and rapid motions alternate. Such kind of periodic behaviour is often called *relaxation oscillation.* One obtains limit cycles even for larger values of $\mu$; in such a case the motion is no longer close to the quasi-steady states.

This example shows that the steady state approximation for rapid subsystems can only be applied after careful scrutiny of the stability properties of the quasi-stationary states of the systems, otherwise there is the danger that one analyses unstable quasi-steady states which do not represent the motion of the slow variables. On the other hand the knowledge of unstable quasi-steady states is important for the understanding of complicated motions of biological systems, which are characterized by discontinuous changes and periodicities.

(ii) *Rapid Reversible Reactions Suppress Catastrophes.* It can be proved that in a dynamic system in which all rapid reactions are reversible metabolic catastrophes cannot occur. We consider the system of reactions

$$(S_1, \ldots, S_n) \xrightarrow[v_1]{} X_1 \underset{w_{-2}}{\overset{w_2}{\rightleftharpoons}} X_2 \xleftarrow[v_3]{} (S_1, \ldots, S_n). \tag{3.99}$$

It consists of the substrates $X_1$ and $X_2$ of the rapid reactions $w_2$ and $w_{-2}$, which are imbedded in a system of slow reactions with the metabolites $S_1 \ldots S_n$. For the kinetics of $w_2$ and $w_{-2}$ one may write, according to Section III.1,

$$w_2 - w_{-2} = \frac{k_2 X_1 - k_{-2} X_2}{\mu} R \tag{3.100}$$

in which $R$ is again the regulating factor, the structure of which depends on the special mechanism of enzymes which catalyze the rapid reactions. $R$ also reflects the influence of the substrates $X_1$ and $X_2$ and that of possible effectors such as the metabolites $S_1$. For the reaction mechanism one obtains the differential equations

$$\frac{dX_1}{dt} = v_1 - \frac{(k_2 X_1 - k_{-2} X_2)}{\mu} R \tag{3.101a}$$

$$\frac{dX_2}{dt} = v_3 + \frac{(k_2 X_1 - k_{-2} X_2)}{\mu} R. \tag{3.101b}$$

By addition and subtraction of these equations the slow and rapid motions are separated in a similar manner as in the example given in Section III.5.(b).(i). If one sets

$$Y_1 = X_1 + X_2; \; Y_2 = X_1 - X_2 \tag{3.102}$$

one obtains

$$\frac{dY_1}{dt} = v_1 + v_3 = \mathbf{g} \tag{3.103a}$$

$$\frac{dY_2}{dt} = v_1 - v_3 - \frac{[k_2(Y_1 + Y_2) - k_{-2}(Y_1 - Y_2)]}{\mu} = \mathbf{G}. \tag{3.103b}$$

Since the factor $\mu$ enters only in Eqn. (3.103b), $Y_1$ represents an additional slow variable to $S_i$ and $Y_2$ a rapid one. If $\mu$ is small the system relaxes to the quasi-steady state surface which is defined by

$$\mathbf{G}(Y_1, Y_2, S_i) = 0. \tag{3.104}$$

The system remains infinitesimally close to this plane if that is stable with respect to the rapid variable $Y_2$, i.e. if

$$\frac{\partial \mathbf{G}}{\partial Y_2} < 0. \tag{3.105}$$

We shall now demonstrate that this stability condition is always fulfilled, if $\mu$ is sufficiently small. From Eqn. (3.103b) it follows for the quasi-steady state plane if $\mu \to 0$

$$Y_2^0 = -\left(\frac{k_2 - k_{-2}}{k_2 + k_{-2}}\right) Y_1^0. \tag{3.106}$$

If one transforms this equation to the original variables $X_1$ and $X_2$, it may be seen that it is identical with the equilibrium ratio

$$\frac{X_2^0}{X_1^0} = \frac{k_2}{k_{-2}}. \tag{3.107}$$

For small but finite $\mu$ we assume $Y_2 = Y_2^0 + \Delta Y_2$; $Y_1 = Y_1^0$ and linearize $\mathbf{G} = 0$ in the neighbourhood of the plane $Y_2^0 = Y_2^0(Y_1^0)$. We obtain for this approximation

$$\Delta Y_2 = \frac{\mu(v_1 - v_3)}{(k_2 + k_{-2})R}. \tag{3.108}$$

In the system of the original variables Eqn. (3.108) is equivalent to

$$\Delta X_1 = -\Delta X_2 = \tau_2 v_{\text{net}}. \tag{3.109}$$

Equation (3.109) originally derived by Hammes and Alberty (1960) represents a simple relationship between the relaxation time $\tau_2$ of the fast enzyme $E_2$, the net flux $v_{\text{net}}$ and $\Delta X_1$, the deviations of the metabolite concentrations in the steady state from their equilibrium. Equation (3.109) has been used by Schimassek *et al.* (1963) for the estimation of the relaxation time of enzymes near to equilibrium. From

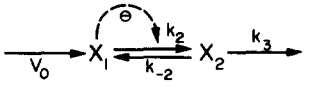
$$\frac{\partial \mathbf{G}}{\partial Y_2} = \frac{\partial(v_1 - v_3)}{\partial Y_2} - \left[\frac{(k_2 + k_{-2})Y_2 + (k_2 - k_{-2})Y_1}{\mu}\right]\frac{\partial R}{\partial Y_2} - \frac{(k_2 + k_{-2})}{\mu}R \tag{3.110}$$

one obtains by the use of Eqns. (3.102) and (3.106)

$$\frac{\partial \mathbf{G}}{\partial Y_2} = -\frac{(k_2 + k_{-2})}{\mu}R + \tfrac{1}{2}(v_1 - v_3)\frac{1}{R}\left(\frac{\partial R}{\partial X_2} - \frac{\partial R}{\partial X_1}\right) + \tfrac{1}{2}\left(-\frac{\partial v_1}{\partial X_2} + \frac{\partial v_1}{\partial X_1} + \frac{\partial v_3}{\partial X_2} - \frac{\partial v_3}{\partial X_1}\right). \tag{3.111}$$

The first term of Eqn. (3.111) is always negative. Since the kinetic equations represent saturation functions, one can presuppose that all other terms in Eqn. (3.111) do not exceed certain finite values. Therefore, one can always obtain a sufficiently small, but finite $\mu$, so that the condition of stability given by Eqn. (3.105) is fulfilled over the entire quasi-steady state plane which is defined by Eqn. (3.104). The concentrations of the substrates of the rapid reversible reaction $X_1$ and $X_2$ are therefore, always close to their equilibrium. Therefore, on variation of the slow variables $S_i$ or $Y_l$ the system will never reach a critical point, at which the rapid reaction veers widely from equilibrium and the system jumps. This means that the rapid reactions are concealed in the motion of the total system.

*Example*: As an example we consider a reaction system depicted in Scheme 8 with a feedforward inhibition of the reversible reaction.




Scheme 8.

The system is described by the following differential equations

$$\frac{dX_1}{dt} = v_0 - \frac{k_2 X_1 - k_{-2} X_2}{(1 + aX_1^n)\mu} \tag{3.112a}$$

$$\frac{dX_2}{dt} = -k_3 X_2 + \frac{k_2 X_1 - k_{-2} X_2}{(1 + aX_1^n)\mu}. \tag{3.112b}$$

From these equations one obtains for the quasi-steady states of the rapid variable $Y_2 = X_1 - X_2$ the curves shown in Fig. 15. It may be seen that for very small values of the parameter $\mu$, i.e. if the reversible reaction is rapid enough, the unstable section $\mathbf{G}^-$ disappears, which had been generated by the feedforward inhibition.

### (d) *The Biological Significance of Time Hierarchies*

The preceding sections have dealt mainly with the methodical problems connected with the existence of time hierarchies in metabolic systems. It was shown that the existence of an epoch of rapid reactions if they are reversible facilitates greatly the theoretical analysis since it permits the description of a dynamic system in the state space of

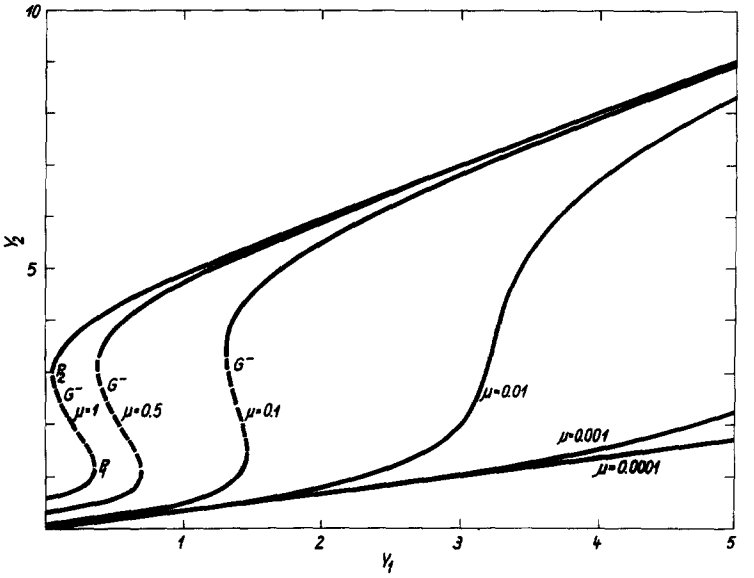



FIG. 15. Disappearance of the multiplicity of the quasi-steady state line by acceleration of the feed-forward inhibited reaction of Scheme 8. Parameter values: $v_0 = 1$, $k_2 = 0.5$, $k_{-2} = 1$, $k_3 = 0.5$, $a = 3$, $q = 4$.

the essential slow variables which is of lower dimensions. In this section we endeavour to bring out the biological significance of time hierarchies. As a rule a metabolic chain consists of many reactions, consistent with the complicated multistep nature of metabolism.

Computer studies have shown (Gardner and Ashby, 1970) that the probability of the instability of a system rises with the number of variables. Therefore the existence of time hierarchies may be an important factor in the stabilization of metabolic systems by virtue of the reduction of the number of dynamically essential variables. Another important feature of biological systems is their self-stabilizing character, which is maintained in the face of many perturbations. With smaller parameters $\mu$, i.e. faster reversible reactions, a point is reached where the quasi-steady state planes on which the essential motions take place become smooth. Under such circumstances the rapid reversible reactions are close to their equilibria, and all parameters which affect the properties of the equilibrium enzymes, and are expressed in the regulating factor $R$, become unimportant for the dynamics of the metabolic system. Or to put it in general terms: the global dynamic properties of the system become structurally stable with respect to the changes of the parameters of the rapid reversible reactions. Therefore the system is determined not only by a lesser number of variables but also by fewer parameters if $\mu$ if sufficiently small.

Such considerations may be of significance for the evolution of biological systems. It is conceivable that the stabilization of metabolic systems during evolution may have been achieved by an optimal adaptation of catalytic efficiencies and concentrations of enzymes to widely different values. The stabilization by widely different rate constants has been proved for the special case of feedback inhibition (see Section III.4.(a).(ii)). The considerations apply not only to the overall reactions of enzymes. Each enzyme catalyzed reaction is composed of many reversible partial "microscopic" reactions, that are characterized by a multitude of parameters, which are the subject of pure enzymology. Only the circumstance that all these microscopic reactions are reversible, and generally much more rapid than the overall reaction, justifies the usual assumption of a steady state for enzyme reactions in metabolic systems and enables one to describe them by few macroscopic kinetic constants. Otherwise the theoretical description of biological systems would be a hopelessly complex task.

One may surmise that during evolution there occurred not only an increase of complexity, as needed for the growing number of biological functions, but also a greater compactness of the systems by virtue of the establishment of widely varying time constants. In such a manner the structural stability of the systems might have increased.

## IV. CONTROL OF STEADY AND TRANSIENT STATES

For the elucidation of the functional behaviour of metabolism it is necessary to evaluate the role of the enzymes for the regulation of the pathways. Control enzymes may be defined by the influence of their parameters either on steady states or transient states. The identification of control enzymes has been a main problem in the investigation of metabolic regulation both in experimental and theoretical work.

### 1. *Identification of Regulatory Enzymes*

For an analysis of the regulation of metabolism a clear and unambiguous definition of the role of an enzyme is essential. We consider a *regulatory enzyme* to be one the properties of which influence either metabolite concentrations or fluxes and which is affected by effectors other than substrates. We distinguish between the control in a steady state and that in a time dependent process. In the latter case a further definition is used: an enzyme is called *time limiting* if it has an influence on the relaxation time of the system.

Similar but not entirely coincident definitions have been given by other authors. Krebs defined *pacemakers* as enzymatic steps the velocities of which are not limited by the substrate concentrations but by the activities or concentrations of the enzymes. The main experimental criterion for the identification of pacemakers was the observation of opposite changes of the velocity of an enzyme and the substrate concentration (Krebs, 1957; Krebs and Kornberg, 1957). Bücher and Rüssmann (1963) criticized the definition mainly because of the finding that glycolytic flux and fructose 6-phosphate change in parallel in skeletal muscle although the phosphofructokinase is obviously an important flux regulator. They defined a *rate limiting* enzyme as one which controls the flux through an enzyme sequence. They recognized the possible existence of several rate limiting enzymes for one pathway such as phosphorylase and phosphofructokinase in muscle. The main experimental criterion for their identification was the deviation of the mass action ratio from equilibrium. The *rate controlling* concept of Higgins (1965) is similar to that of Bücher and Rüssmann (1963). Higgins proposed a quantitative expression for the influence of an enzyme on the flux, the *control strength.*

All these concepts consider only the control of the flux. Newsholme and Start (1973) and Rolleston (1972) defined a regulatory enzyme more broadly as one which is influenced by factors other than substrates and affects either flux or metabolites. They specified that the reaction must be catalyzed by a *non-equilibrium* enzyme. Although it is true that regulation by an enzyme implies deviation from equilibrium one may object that the term "non-equilibrium" does not only refer to an intrinsic property of an enzyme but rather may indicate the conditions under which it operates. It would be preferable to restrict the term to those enzymes which catalyze reactions that are far from equilibrium under all circumstances in the cell. Otherwise, the definition of a regulatory enzyme is identical with the one here proposed.

Weber (1974) introduced the concept of *key enzymes.* He lists fourteen characteristic features of such enzymes, including rate limitation, low activity, catalysis of quasi-irreversible reactions, their allosteric regulation by feedback and multiple control signals and their frequent position in an enzyme sequence either at the beginning or at the end. He makes no attempt to indicate whether all of these features are necessary or sufficient properties. Thus, his concept falls short of an exact definition.

How to identify experimentally a regulatory enzyme? Several procedures have been proposed (e.g. Newsholme and Start, 1973; Hales, 1967) and some of the definitions given above are closely connected with the experimental methods (e.g. pacemakers, rate limiting enzymes).

The most general and widely acknowledged criterion is the deviation of the mass action ratio from the equilibrium constant. Enzymes which catalyze reactions far from equilibrium regulate in general the concentrations of the metabolites but not necessarily the flux through an enzymatic chain (see Section IV.3).

It has been assumed that the enzymes with the lowest maximal velocities are regulatory. Slowness is indeed an important although not sufficient characteristic of a regulatory enzyme and there is an intimate relation between the slowness of an enzyme and the deviation of the mass action ratio from equilibrium (Section IV.3.(a)). However, the maximal velocities determined under optimal conditions *in vitro* do not adequately characterize the slowness of an enzyme in metabolism. Generally, the activities *in vivo* lie far below the $V_{max}$-values of the enzymes owing to the action of effectors in the cell. Even in the simplest case of a linear dependence of the enzyme velocity on the substrate concentration the first order rate constant $V_{max}/K_m$ would be preferable to $V_{max}$ alone. In agreement with this theoretical argument, aldolase and enolase have rather low $V_{max}$-values but are not regulatory in glycolysis, whereas the pyruvate kinase influences the concentrations of many metabolites although it has a high $V_{max}$-value (Newsholme and Start, 1973).

In all concepts the influence of effectors other than substrates is considered as an indication of a regulatory enzyme. Krebs (1957) noted that an activation of the flux through an enzyme with a simultaneous decrease of its substrate concentration should indicate the action of an activator on this enzyme. Chance *et al.* (1958) took into account both the changes of substrates and products in the *crossover theorem.* In many cases both procedures fail to identify or falsely indicate interactions of enzymes with effectors (see Section IV.3.(b)). The *fault theorem* by Higgins (1974) is an extension of the crossover theorem in which the flux changes are included. Although the application of this theorem does not give rise to erroneous conclusions it does not identify all interaction sites. A preferable quantitative theorem has been proposed (Heinrich and Rapoport, 1974b) which is applicable if the rate laws of the enzymes are known (see Section IV.3.(b)).

The investigation of the influence of effectors on purified enzymes has been a frequent method of identification of regulatory enzymes. The results can only be applied to the conditions in the cell if the influence of the effectors *in vivo* has been proved. There are many examples of allosteric enzymes which have no regulatory function in the cell (e.g. glyceraldehydephosphate dehydrogenase; Conway and Koshland, 1968; Kirschner *et al.*, 1966). The conditions in the cell are better approached by studies on permeabilized cells (Sols and Marco, 1970; Sols *et al.*, 1973) in which the *in situ* kinetics is measured.

Another method for the identification of regulatory enzymes is the use of reconstituted enzyme systems (Gatt and Racker, 1959a,b; Uyeda and Racker, 1956; Lo *et al.*, 1968; Gosálvez *et al.*, 1974; Scopes, 1974) which should allow the study of the coordinated control of several enzymes.

An important method for the identification of the slow enzymes in a system are relaxation experiments. After perturbation of a metabolic system fast reversible enzymes will establish a near equilibrium within a short period of time while the time limiting enzymes will determine the relaxation of the entire system and will be far from equilibrium. Relaxation experiments may require some mathematical effort for their full evaluation but yield more information than other methods.

Finally, the position of an enzyme in a sequence of reactions is of importance for its regulatory role (Section IV.2.(d)). The first enzyme in a chain or one after a branching point is predestined for the control in the steady state. Such a general consideration provides a guideline for the experimental investigation.

### 2. *Control of Steady States*

The role of an enzyme in a steady state must be distinguished from that in a time dependent process. In considering the control of a steady state the question is asked which enzymes determine the fluxes and the metabolite concentrations. We shall consider

solely stable steady states which are the only ones which may be physiologically relevant. Mathematically, the control is expressed in form of control parameters which give the variation of the flux or of the metabolite concentrations after a differentially small change of the parameters. Thus, the control parameters are properties of a given steady state of the system. Owing to the linearization involved they may not represent faithfully the nondifferential large variations observed in real experiments. The role of an enzyme depends on two factors: firstly, its effect on the flux and the metabolite concentrations and secondly, the strength of the effectors acting on it. Therefore, the influence of effectors on the enzymes has to be included in the considerations.

(a) *Definitions of Control Parameters*

Two cardinal terms were proposed to describe the influence of an enzyme on flux and metabolites (Heinrich and Rapoport, 1974a):

(i) The control strength $C_i$ which gives the relative changes of the net flux $v_g$ by relative variations of the activity $v_i$ of an individual enzyme $E_i$

$$C_i = \frac{\partial \ln v_g}{\partial \ln v_i} = \frac{\dfrac{\partial v_g}{v_g}}{\dfrac{\partial v_i}{v_i}}. \tag{4.1}$$

The control strength may assume any value. $C_i = 0$ means that the enzyme has no influence on the total flux. A control strength greater than unity means that the enzyme has a greater than linear influence on the total flux, a negative value, that an activation of this enzyme leads to a decrease of the total flux.
A previous definition of the control strength has been given by Higgins (1965). His expression was

$$C_i = \frac{\partial v_g}{\partial p} \tag{4.2}$$

which is dependent on the choice of the parameter $p$ and is not dimensionless. The present definition can be written as

$$C_i = v_i \frac{\partial v_g}{\partial p} \Big/ v_g \frac{\partial v_i}{\partial p} \tag{4.3}$$

and has the advantages that: 1, it is independent of the choice of the parameter $p$; 2, it is dimensionless; and 3, it is applicable for branched pathways in which $v_i$ may be unequal to $v_g$. A suitable choice of the parameter $p$ is obviously the concentration of an enzyme since the velocity is proportional to it if the substrate concentration is in large excess. In that case the formula (4.3) becomes

$$C_i = \frac{[E_i]}{v_g} \frac{\partial v_g}{\partial [E_i]}, \tag{4.4}$$

where $[E_i]$ denotes the enzyme concentration. This expression has been used to measure the control strength experimentally in erythrocyte glycolysis (Reimann *et al.*, 1975). The definition given above is, however, broader since it is also applicable if the enzyme and substrate concentrations are of equal magnitude. Then, the velocity of the enzyme is not proportional to the enzyme concentration.
It has been criticized (Ottaway, 1976) that the logarithmic definition (Eqn. (4.1)) prohibits its use if negative flux changes occur in response to activations of enzymes. This is obviously a misinterpretation since the logarithmic form is only an abbreviated expression of the fact that percentual changes are compared. The definition (4.1) of the control strength is identical with that of the *sensitivity coefficient* of Kacser and Burns (1973).

(ii) The elements of the *control matrix* $S_{ij}$ give the relative changes of the metabolite concentrations $S_i$ by relative variations of the enzyme activity $v_j$

$$S_{ij} = \frac{\partial \ln S_i}{\partial \ln v_j}. \tag{4.5}$$

$S_{ij} > 0$ means that activation of the enzyme leads to an increase of the concentration of the metabolite and $S_{ij} < 0$ to a decrease. In all cases a given steady state is compared with a new one after differential perturbation of enzyme parameters.

(b) *The Influence of Effectors; The Effector Strength*

The influence of an effector $F_k$ of an enzyme $E_i$ on the net flux and the metabolite concentrations of the entire chain may be described as follows if only small changes $\Delta F_k$ are considered (Heinrich and Rapoport, 1974a)

$$4v_g = \frac{\partial \ln v_g}{\partial \ln v_i} \frac{\partial \ln v_i}{\partial \ln F_k} \Delta F_k \equiv C_i X_{ik} \Delta F_k \tag{4.6}$$

and

$$\Delta S_j = \frac{\partial \ln S_j}{\partial \ln v_i} \frac{\partial \ln v_i}{\partial \ln F_k} \Delta F_k \equiv S_{ji} X_{ik} \Delta F_k. \tag{4.7}$$

$X_{ik}$ is called the effector strength. It is a measure of the influence of an effector on the kinetic properties of the isolated enzyme $E_i$. Analysis of Eqns. (4.6) and (4.7) shows that there are two different requirements for an enzyme to be of regulatory importance with respect to effectors: first, the enzyme must control metabolites or the flux (large values of $S_{ij}$ and $C_i$ respectively) and secondly, it must be the target of effectors. The effector strengths can be calculated easily by differentiation of the rate laws for an enzyme with respect to the effector concentration. Some simple examples are given in Table 3. The essential conclusions to be drawn from Table 3 are that cooperative enzymes have greater effector strengths compared to Michaelis-type enzymes and that their sensitivities to allosteric effectors are greatest at the lowest substrate concentrations. It is possible that the most adaptive property of allosteric enzymes is the higher sensitivity towards effectors rather than the sigmoidal response to substrates which is prominent at higher substrate concentrations and has hitherto attracted most of the attention.

(c) *Calculation Procedures for the Control Parameters*

The calculation of the control parameters by direct differentiation according to Eqn. (4.1) is only possible for simple systems for which analytical expressions for the metabolite concentrations and the flux can be obtained (see Heinrich and Rapoport, 1974a). Generally, this is not possible for nonlinear systems. In this case we go back to the

TABLE 3. EFFECTOR STRENGTHS FOR A COMPETITIVE, NON-COMPETITIVE OR ALLOSTERIC INHIBITOR

| Inhibition | Rate law | Expression for the effector strength |
|---|---|---|
| competitive | $v = V_{max} \frac{S/K_m}{1 + S/K_m + I/K_i}$ | $\frac{I/K_i}{1 + S/K_m + I/K_i}$ |
| non-competitive | $v = \frac{V_{max} S/K_m}{(1 + I/K_i)(1 + S/K_m)}$ | $\frac{I/K_i}{1 + I/K_i}$ |
| allosteric (c.f. model of Monod *et al.* (1965)) | $v = \frac{V_{max} S/K_m}{(1 + S/K_m)\left(1 + \frac{L(1 + I/K_i)^n}{(1 + S/K_m)^n}\right)}$ | $\frac{nI/K_i L \frac{(1 + I/K_i)^n}{(1 + S/K_m)^n}}{(1 + I/K_i)\left(1 \frac{L(1 + I/K_i)^n}{(1 + S/K_m)^n}\right)}$ |

steady state condition (Eqn. (3.8)) and consider small changes in the velocities of an enzyme $E_r$ (Heinrich and Rapoport, 1975):

$$f_i(v_r + \Delta v_r) \sim \sum_{j=1}^{m} \frac{\partial f_i}{\partial S_j} \mathrm{d}S_j + \frac{\partial f_i}{\partial v_r} \mathrm{d}v_r = 0. \tag{4.8}$$

Rearrangement of this equation gives

$$\sum_{l=1}^{n} \sum_{j=1}^{m} c_{il} v_l \frac{\partial \ln v_l}{\partial \ln S_j} \frac{\partial \ln S_j}{\partial \ln v_r} + \sum_{l=1}^{n} c_{il} v_l \frac{\partial \ln v_l}{\partial \ln v_r} = 0 \tag{4.9}$$

$$(i = 1, \ldots, m)$$
$$(r = 1, \ldots, n)$$

where $c_{il}$ are the elements of the stoichiometric matrix defined in Section III.1. By use of the abbreviations for the effector strength and the control matrix we obtain, from Eqn. (4.9),

$$\sum_{l=1}^{n} \sum_{j=1}^{m} c_{il} v_l X_{lj} S_{jr} + \sum_{l=1}^{n} c_{il} v_l \delta_{lr} = 0. \tag{4.10}$$

The fluxes and the effector strengths can be calculated from the steady state equations and the rate laws of the isolated enzymes, respectively. The matrix $c_{il}$ is known from the stoichiometric structure of the system. Thus, the elements of the control matrix $S_{jr}$ may be obtained by solving the $n \times m$ equations of type (4.10). If the control matrix is known the control strengths of the enzymes can be obtained by use of the equation

$$C_i = \frac{\partial \ln v_g}{\partial \ln v_i} = \frac{1}{v_g} \left( \sum_{j=1}^{n} v_j \delta_{ij} + \sum_{j=1}^{n} \sum_{k=1}^{m} v_j X_{jk} S_{ki} \right). \tag{4.11}$$

The sum over $j$ contains all fluxes which contribute to the total flux.

The calculation of the control strengths may be simplified by application of the following fundamental theorems.

(i) The sum of the control strengths is equal to unity.

$$\sum_{i=1}^{n} C_i = 1. \tag{4.12}$$

This summation theorem expresses the fact that a simultaneous activation or inhibition of all reaction velocities by the same factor leads to an equal response in the total flux and does not change the metabolite concentrations.

(ii) Kacser and Burns (1973) proved a theorem which connects the control strengths with the effector strengths of the metabolites

$$\sum_{i=1}^{n} C_i X_{ik} = 0 \qquad (k = 1, \ldots, m). \tag{4.13}$$

The theorem follows from the consideration that differential changes in the concentration of a metabolite $S_k$ can be compensated by variations of the kinetic parameters of the enzymes which are influenced by this metabolite so that the flux remains unaltered.

For metabolic systems in which the number of enzymes exceeds the number of metabolites only by one (e.g. linear enzymatic chains), theorems (i) and (ii) suffice for the calculation of the control strengths. In case of branches in the system the number of enzymes can exceed that of the metabolites by more than one and the control strengths of at least $(n - (m + 1))$ enzymes must be calculated by means of Eqns. (4.10) and (4.11). The remaining control strengths can be obtained by the theorems (i) and (ii).

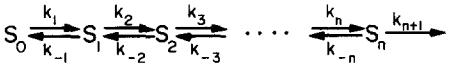
Ottaway (1976) claimed that the summation theorem for the control strengths (4.12) does not hold if the concentrations of the enzymes are not much lower than those of the substrates. This is not correct, since the derivation of the theorem is independent of the kinetics of the enzymes if the general definition of the control strength (4.1) is used. His argument is based on the special definition (4.4) which is not applicable if the substrate is not in large excess.

The influence of small changes of different parameters on the flux and the concentrations of metabolites may be studied also by solving the steady state Eqns. (3.8) of the model on computers. Thereby the control parameters are also obtained. Such an analysis is sometimes called *sensitivity analysis* (Anderson *et al.*, 1971). The calculation procedure of the control parameters by Eqns. (4.10) and (4.11) does not only work with fluxes obtained by a mathematical model but also with steady state fluxes derived from experiments.

The elements of the control matrix may also be valuable for modeling purposes (Garfinkel *et al.*, 1976). The metabolites which are most sensitive to the fluxes should be measured experimentally while those with low sensitivities are less important for fixing the model parameters.

*(d) Control in Linear Enzymatic Chains*

The linear enzymatic chain is a sequence of first order reactions (Scheme 9).



Scheme 9.

The following expression is obtained for the flux through the entire chain as a function of the relaxation times and the equilibrium constants of the enzymes (Heinrich and Rapoport, 1974a)

$$v_g = \frac{S_0 \prod_{i=1}^{n} q_i}{\tau_{n+1} + \sum_{k=1}^{n} \tau_k (1 + q_k) \prod_{m=k+1}^{n} q_m}. \tag{4.14}$$

By differentiation of this equation one obtains for the control strengths

$$C_i = \frac{\dfrac{\partial v_g}{\partial \tau_i}}{\dfrac{\partial v_i}{\partial \tau_i}} = \frac{\tau_i (1 + q_i) \prod_{j=i+1}^{n} q_j}{\tau_{n+1} + \sum_{k=1}^{n} \tau_k (1 + q_k) \prod_{j=1}^{n} q_j} \tag{4.15}$$

The control strengths in this case may range from 0 to 1. For $q_i = 1$ one gets, from Eqn. (4.15),

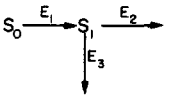
$$C_i = \frac{2\tau_i}{\tau_{n+1} + 2 \sum_{k=1}^{n} \tau_k}. \tag{4.16}$$

Thus, the control strength of the enzyme $E_i$ bears a direct relation to the relaxation time, i.e. the slowest enzymes have the greatest influence on the flux. Reactions which are very fast ($\tau_i \to 0$) have no influence on the flux. Analysis of Eqn. (4.15) shows that enzymes $E_i$ beyond a quasi-irreversible reaction $E_s (q_s \to \infty, i > s)$ have no influence on the flux ($C_i = 0$) even if they are slow. In particular, if the first enzyme is quasi-irreversible it alone will determine the steady state flux through the chain if no feedback signals exist from the later steps. This statement is also valid for systems with enzymes which respond nonlinearly to substrate concentrations. This has not been fully recognized in the literature. Experimenters have often ascribed flux control to enzymes beyond

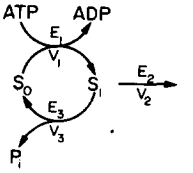
quasi-irreversible steps without proving that feedback signals exist (Maitra and Chance, 1965; Williamson, 1965; Ghosh and Sloviter, 1973; Gosálvez *et al.*, 1975). Simulators have built rather complicated models to reach the above mentioned almost trivial result which could have been easily anticipated (Achs *et al.*, 1971; Ottaway, 1976).

(e) *Control in Substrate Cycles and Branched Pathways*

Substrate cycling occurs in the glycolytic chain at the levels of the hexokinase-glucose 6-phosphatase, phosphofructokinase-fructose 1,6-bisphosphatase and probably at the pyruvate kinase-phosphoenolpyruvate carboxykinase (Katz and Rognstad, 1976). The apparent wastefulness of the cycles has lead to speculations on their role in the regulation of metabolism. Evidence has been given that the generation of heat by hydrolysis of ATP is of importance for the bumble bee (Clark *et al.*, 1973; Bloxham *et al.*, 1973). A stabilizing effect by futile cycles on the ATP-level has been proposed by Rapoport *et al.* (1976a). For instance, a decrease of the ATP-level and concomitant increase of the concentrations of AMP and ADP would activate the phosphofructokinase reaction and inhibit the fructose 1,6-bisphosphatase reaction. Thus, futile cycling would be decreased and so the ATP-waste diminished. In a further hypothesis there is assumed an amplifying effect by futile cycles on the flux control (Newsholme and Gevers, 1967; Scrutton and Utter, 1968; Hue and Hers, 1974). This hypothesis will be discussed on the basis of the Schemes 10 and 11. The Scheme 11 for a futile cycle becomes virtually identical with that for a branched pathway (Scheme 10) if ATP, ADP and $P_i$ have




Scheme 10.




Scheme 11.

no influence on the reaction rates and if $S_0$ is held constant. If the enzymes $E_2$ and $E_3$ are linearly dependent on their substrate $S_1$ we get by application of the theorems (i) and (ii) of Section IV.2.(c) for the control strengths with respect to the outflow $v_2$

$$C_2 + C_3 = 0, \; C_1 = 1.$$

Furthermore, Eqns. (4.10) and (4.11) yield

$$C_2 = -C_3 = \frac{v_3}{v_2 + v_3} < 1. \tag{4.17}$$

Obviously, in this case all enzymes have control strengths between 0 and 1 and there is no amplifying effect. However, if $E_3$ is saturated by the concentration of its substrate $S_1$ one obtains

$$C_2 = 0, \quad C_1 + C_3 = 1 \tag{4.18}$$

and by Eqns. (4.10) and (4.11)

$$C_3 = -\frac{v_3}{v_1 - v_3} = -\frac{v_3}{v_2}. \tag{4.19}$$

Now $C_1$ and $C_3$ may assume any value. They become very large if the net flux $v_2$ is small compared to $v_1$ and $v_3$. For futile cycles this means that an amplifying effect is possible provided that the cycle flux is greater than the net outflux. The regulatory advantage is paid for a high ATP-waste. An amplifying effect is only possible if the backward reaction $S_1 \rightarrow S_0$ depends only weakly or not at all on the concentration of the substrate $S_1$ (see Eqns. (4.17) and (4.19)). If one assumes $S_0$ to be produced

by a constant influx (Newholme, 1975) the net outflux in the steady state is independent from the activation state of the cycle enzymes which only affects the cycle flux and the ATP-waste and there is no amplification.

### 3. *Changes of Metabolite Concentrations as Indicators of Control Sites in Enzymatic Systems; A Critical Discussion*

#### (a) *The Deviation of the Mass Action Ratio from the Equilibrium Constant*

The relation between the velocity $v_i$ of an enzyme $E_i$ and the concentrations of its reactants, given in Eqn. (3.4), may also be written in the following way

$$\frac{\Gamma_i}{q_i} = 1 - \frac{v_i}{k_i R_i(S_k) \prod_{j,\,substrates} S_j}. \tag{4.20}$$

In this equation $\Gamma_i$ is the mass action ratio, $q_i$ the equilibrium constant of the enzyme $E_i$ and $R_i$ the regulating factor. We shall discuss here the conclusions which may be drawn from this relation for an enzyme which is part of a metabolic system in the steady state. At first sight it would appear that Eqn. (4.20) indicates a direct relation between the deviation from equilibrium and the flux through an enzyme. However, this relationship is only indirect since it is also influenced by the concentrations of the reactants. Changes of flux which are caused by alteration of the kinetic parameters generally also are accompanied by variations of the concentrations of the metabolites. Therefore Eqn. (4.20) does not permit to draw definite conclusions.

This may be illustrated by the following simple reaction system

$$\xrightarrow{v_0} S_1 \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} S_2 \xrightarrow{k_2}$$

where the reactions are described by first order rate constants and the input $v_0$ is independent from $S_1$ and $S_2$. From Eqn. (4.20) we obtain

$$\frac{\Gamma_1}{q_1} = 1 - \frac{v_0}{k_1 S_1}. \tag{4.21}$$

Since in the steady state

$$S_1 = \left(\frac{k_{-1} + k_2}{k_1 k_2}\right) v_0 \tag{4.22}$$

one obtains

$$\frac{\Gamma_1}{q_1} = \frac{1}{1 + (k_2/k_{-1})}. \tag{4.23}$$

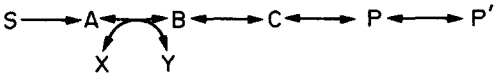
In this case the deviation of the mass action ratio from the equilibrium constant is entirely independent from the flux and is only a function of the kinetic parameters of the enzymes $E_1$ and $E_2$. Heinrich and Rapoport (1974a) proved that in linear enzyme systems which contain only reactions of the first order the mass action ratio of an enzyme is generally independent from the kinetic parameters of all enzymes preceding it. Keeping in mind that the first enzymes exert the main control of the flux in a linear chain one may conclude that a direct connection between the mass action ratio and the control strength of an enzyme, as often claimed in the literature, does not exist. Enzymes catalyzing reactions which deviate greatly from equilibrium need not exert any control on the metabolic flux (see Section IV.2.(d)). The deviation of the mass action ratio from the equilibrium constant indicates only that the enzyme has an influence on the concentrations of metabolites, i.e. it has elements in the control matrix which are unequal to zero.

If one replaces $k_{-1}$ in Eqn. (4.23) by the relaxation time and the equilibrium constant (see Eqns. (3.3) and 3.51b)) one obtains

$$\frac{\Gamma_1}{q_1} = \frac{1}{1 + k_2\tau_1(1 + q_1)}. \tag{4.24}$$

It may be seen that the larger the equilibrium constant of the enzyme the greater is the deviation of the reaction from equilibrium. This has been proved generally for linear systems of reactions (Heinrich and Rapoport, 1974a). It explains why many reactions with ATP as reaction partner show large deviations from the equilibrium constant (Minakami and Yoshikawa, 1966).

From Eqns. (4.15) and (4.24) it follows that enzymes which are so fast that they do not show deviations from equilibrium (small $\tau$-values) also cannot influence the flux. This statement is in contrast to the generally accepted possibility of rate control by equilibrium enzymes (Hales, 1967; Krebs, 1969; Newholme and Start, 1973). The argumentation in the literature is illustrated by the following model (Newsholme and Start, 1973) (see Scheme 12).




Scheme 12.

The assumptions implicit in this model are as follows:

(i) All reactions between $A$ and $P$ are close to equilibrium.
(ii) The product $P$ is removed to $P'$ by a non-equilibrium reaction at a rate which is proportional to the concentration of $P$.
(iii) The compound $A$ is produced by a non-equilibrium reaction which is controlled in such a way that the concentration of $A$ remains constant.
(iv) $X$ and $Y$ are cofactors for the reaction $A \leftrightarrow B$ and the total cofactor concentration $X + Y$ remains constant.

If the concentration of $X$ is increased and that of $Y$ correspondingly decreased, an increase of $B$ and consequently of $P$ results. Therefore the flux to $P'$ is increased. It is concluded that the pathway $S \rightarrow P'$ has responded to the change in the regulator concentrations ($X$ and $Y$) via a reaction which is near to equilibrium.

The error in this argumentation is due to assumption (iii). For $A$ to remain constant the reaction $S \rightarrow A$ must be activated. The changes of $X$ and $Y$ can change the flux through the pathway only if a feedback signal exists to the reaction producing $A$. Therefore the flux regulation is actually exerted by a non-equilibrium enzyme. On the other hand, the example demonstrates that changes of metabolite concentrations can be produced by variations of the partners of a near-equilibrium reaction. This is, however, no regulation by, but rather via, an equilibrium enzyme.

The equilibria undoubtedly may play an important role in the transmission of the state of one pathway to another. The consequences depend on the effects of the partners of an equilibrium on the regulatory enzymes. Equilibria can also be used as diagnostica for the state of a system (Krebs, 1969; Krebs and Veech, 1969; Gumaa *et al.*, 1971; Safer and Williamson, 1973).

(b) *Identification of Interaction Sites of Effectors with an Enzymatic Chain; the Crossover- and Fault-Theorems*

A central problem in the study of metabolic systems is the identification of interaction sites of external effectors. The experimental procedure is most often the investigation of the response of a system to external effectors as indicated by changes of metabolites. For this purpose commonly the *crossover theorem* is applied which has been formulated originally by Chance and coworkers (Chance and Williams, 1955a,b; 1965a,b; Chance *et al.*, 1958) and utilized by them for the identification of the phosphorylation sites in the respiratory chain. Holmes (1959) was the first to prove the crossover theorem

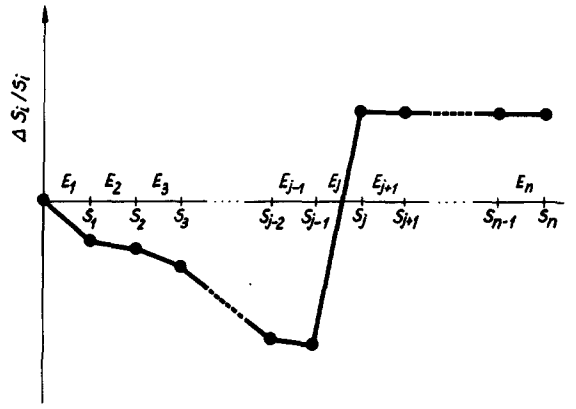



FIG. 16. Crossover plot for a linear enzymatic chain. The graph gives the relative changes of the metabolite concentrations versus the sequence of the metabolites after interaction of an activator with the enzyme $E_j$.

for a number of simple chemical chains of reactions. In its simplest form the classical crossover theorem can be stated in the following way: *The changes of the steady state concentrations of the metabolites beyond and before an enzyme which is influenced by an effector have different signs.* For the detection of crossovers a graph is constructed by plotting the percentual changes of the concentrations of the intermediate metabolites after the addition of an effector versus their sequence in an enzymatic chain. This graph is called a *crossover-plot* (Fig. 16). Points at which the graph crosses the axis are called *crossover-points.* Theoretically, the crossover theorem is based on the *fault-theorem* which has been analyzed mainly by Higgins (1974)*. It is derived from the *concept of generalized reactions*, for which only the monotonicity properties of the rate equations are required. The generalized rate equation for the reaction

$$\text{substrates} \underset{\uparrow \ominus \text{ inhibitors}}{\overset{\downarrow \oplus \text{ activators}}{\rightleftharpoons}} \text{products}$$

may be written as

$$v = v(\text{substrates, activators/products, inhibitors}). \tag{4.25}$$

Equation (4.25) is an abbreviated formulation of the fact that elevations of the concentrations of substrates and activators increase the velocity whereas elevations of the concentrations of products and inhibitors decrease it. Table 4 gives for a generalized monomolecular reaction the signs of the changes of the reactants and the flux which may be experimentally measured. All changes except those indicated as "fault" are consistent with the monotonicity properties of the rate equation. If a fault occurs, either the rate equation is wrong or incomplete, i.e. some other agent is affecting the rate of that reaction. For instance, if one observes that the net flux through the reaction diminishes whereas the substrate increases and the product decreases it may be concluded that an additional inhibitor must be interacting with the enzyme. The fault theorem can be applied regardless of the complexity of the entire enzyme system. It can be used with time dependent processes provided that the individual net reaction velocities can be measured. Compared with the crossover theorem it does not give rise to erroneous assumptions of interaction sites. However, owing to its qualitative nature the fault theorem does not identify all interaction sites. If there is more than one interaction site the weaker ones may fail to show up as faults.

*Higgins has written up a detailed investigation on the crossover and fault theorems (personal communication) but to our knowledge has not published it.

TABLE 4. CHANGES OF THE FLUX $v$ AND OF THE CONCENTRATIONS OF THE SUBSTRATE $S$ AND OF THE PRODUCT $P$ FOR A GENERALIZED MONOMOLECULAR REACTION $S \rightleftarrows P$

| $\Delta v$ | $\Delta S$ | $mP$ | |
|---|---|---|---|
| + | + | + | |
| + | + | − | |
| + | − | − | |
| + | − | + | fault |
| − | + | + | |
| − | + | − | fault |
| − | − | − | |
| − | − | + | |

(+) signs indicate an increase and (−) signs indicate a decrease. Changes of the metabolite concentrations which are not denoted by "fault" are compatible with the change of the flux.

Another method for the determination of interaction sites which uses a quantitative formulation of the rate equations with respect to substrates, products and known activators and inhibitors was proposed by Heinrich and Rapoport (1974b). If the insertion of the experimental changes of flux and concentrations leads to changed values of the kinetic parameters of the enzymes an interaction with an effector is indicated. The quantitative formulation allows the identification of effector interactions even if the extreme case of a fault is not observed.

As an example of the application of the fault and crossover theorems we consider an enzyme sequence and the interaction of an activator $A_1$ at one site (Fig. 17). It is presupposed that all reactions except for the final step are reversible. The concentration of the first metabolite is assumed to be constant. After the interaction of the activator the metabolite concentrations change and assume new steady state values. The signs in Fig. 17 indicate the direction of the changes of the metabolites. It can be easily demonstrated in the case of validity of the generalized rate law (4.25) for the enzymes of the sequence that the concentrations of all metabolites beyond the interaction site increase, while those of the metabolites preceding it decrease. In as much as the net flux through the enzymatic chain increases, a crossover appears which is a fault (see Table 4) because the influence of the activators had not been specifically included in the generalized rate law. In this case, therefore, the occurrence of a crossover identifies the interaction site of an effector. However, the crossover theorem may be quite misleading in more complicated situations, e.g. with an effector acting at more than one site. A crossover may not appear at an affected enzyme, or *pseudo-crossovers* may occur at unaffected enzymes.

As an example, for these possibilities we consider again a linear enzyme sequence and the interaction of an activator $A_2$ at two different sites (Fig. 17). It is seen that three different crossover patterns may be obtained. Which possibility is observed depends on the ratio of the effector strengths of the activator at the two different sites and the control parameters of the enzymes (Heinrich and Rapoport, 1974b). If one of the

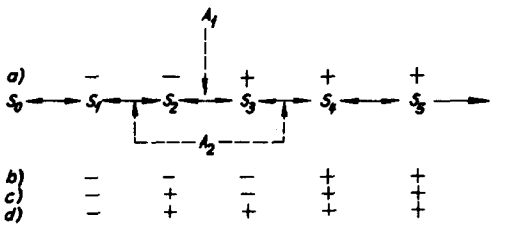



FIG. 17. Crossover pattern for the interaction of activators with a linear enzymatic chain. The action of the activator $A_1$ produces the pattern (a). The activator $A_2$ acting simultaneously on two enzymes produces one of the patterns (b)–(d).

interactions is much stronger than the other either pattern (b) or (d) will be observed. There will be only one crossover at that enzyme which is more strongly affected. If the effector strengths are comparable crossovers may appear at both sites if the enzymes between them are slow with control strengths unequal to zero. In this case (pattern (c) in Fig. 17) a third crossover occurs which is not a fault as may be seen from Table 4.

The theorem may be also quite misleading if systems involving conservation quantities are considered. Figure 18 shows an enzymatic system which contains a loop. It may be seen easily that for all changes of enzyme parameters the sum of the metabolites concentrations which belong to the loop must be conserved. This means that an increase of some metabolites of the cycle must be compensated by a decrease of others. Figure 18 shows the changes of the metabolite concentrations for the case that the first enzyme which does not belong to the cycle is activated. It is seen that two pseudo-crossovers occur within the loop at the enzymes $E_2$ and $E_5$. Pseudo-crossovers may also appear in systems involving the action of inner effectors (feedback activation or inhibition etc.; Higgins, J. personal communication). Furthermore, the limitations of the application of the crossover theorem are apparent if one considers systems where metal ions (e.g. $Mg^{2+}$) form complexes with the metabolites (Heinrich and Rapoport, 1974b).

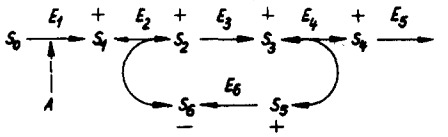



FIG. 18. Crossover pattern for an enzymatic system containing a loop. The activator $A$ acts on the first enzyme $E_1$. At the enzyme $E_6$ a crossover is produced which is not indicative for an interaction of the enzyme with an effector.

The essential conclusion of the work by Higgins (1974) and Heinrich and Rapoport (1974b) is that experimenters should be warned against the uncritical use of the crossover theorem. It may be applicable for systems which are well known for which a new interacting agent is tested. Usually, the fault theorem and if possible, its quantitative extension, is to be preferred. Although the crossover theorem was originally developed for transitions from one steady state to another, it was also used for time dependent processes (Williamson, 1965) by comparing the metabolite concentrations at two different times. This extension lacks any theoretical basis. Crossovers in time dependent processes may occur without any action of an effector e.g. just by adding an intermediate and observing its degradation through a sequence of reactions. Crossovers also generally appear in oscillating reactions.

### 4. *Control of Transient Processes*

In this section we are concerned with the question: what determines the overall rate of the changes of metabolites during relaxation processes? In order to proceed with the answer one has to define a transition time. Although the reciprocals of the eigenvalues do have the dimension of time, they are not suitable in this context, since they define the relaxation of the whole system rather than that of individual metabolites. A suitable measure for the characterization of the rate of transition of a metabolite to a stable steady state has been proposed by Heinrich and Rapoport (1975). A transition time for the metabolite $S_i$ is defined as follows

$$\hat{\tau}_i = \frac{\int_0^\infty t\sigma_i(t)\,dt}{\int_0^\infty \sigma_i(t)\,dt} = \frac{T_i}{I_i} \tag{4.26}$$

where the perturbation terms $\sigma_i(t) = S_i(t) - S_i^0$($S_i^0$—final steady state value of $S_i$) are called in this connection *relaxation functions*. For $t \to \infty$ the terms $\sigma_i(t)$ tends to zero.

The relaxation functions may be considered as weighting factors of the time $t$. The applicability of expression (4.26) is limited by the requirement that the relaxation functions do not change their sign during the process. Otherwise, negative weighting factors would occur in the integrals. Therefore, this definition is meaningless for processes which involve damped oscillations, overshoots or undershoots. The *transient control* $K_{ij}$ of an enzyme $E_j$ may be defined by the influence of its activity $v_j$ on the transition time of the metabolite $S_i$

$$K_{ij} = -\frac{\partial \ln \hat{\tau}_i}{\partial \ln v_j}. \tag{4.27}$$

It is positive, if an activation of the enzyme leads to a decrease of the transition time, otherwise it is negative. The following summation theorem holds

$$\sum_{j=1}^{n} K_{ij} = 1. \tag{4.28}$$

It is based on the consideration that a simultaneous activation or inhibition of all enzymes by the same factor leads to equal changes in the transition times of all metabolites. Similar considerations have led to the summation theorem for the control strengths (Eqn. (4.12)).

A simple calculation procedure for $\hat{\tau}_i$ can be employed which does not involve the integration of the differential equations if only small deviations from the steady state are considered. Integration of the linearized equation (3.9) yields

$$\int_0^\infty \frac{\mathrm{d}\sigma_i}{\mathrm{d}t}\,\mathrm{d}t = -\sigma_i(0) = \sum_k A_{ik}\int_0^\infty \sigma_k\,\mathrm{d}t \equiv \sum_k A_{ik}I_k. \tag{4.29}$$

Furthermore, using well-known integration rules we have

$$\int_0^\infty \sigma_i\,\mathrm{d}t = t\sigma_i(t)\Big|_0^\infty - \int_0^\infty t\frac{\mathrm{d}\sigma_i}{\mathrm{d}t} = -\sum_k A_{ik}\int_0^\infty t\sigma_k\,\mathrm{d}t. \tag{4.30}$$

The terms $t\sigma_i(t)/_0^\infty$ may be neglected since $\sigma_i(t)$ decreases more than $1/t$ with increasing time. Thus, the integrals $I_i$ and $T_i$ which enter Eqn. (4.26) can be calculated by solving the following linear equation system

$$-\sigma_i(0) = \sum_{k=1}^{n} A_{ik}I_k \tag{4.31}$$

$$-I_i = \sum_{k=1}^{n} A_{ik}T_k. \tag{4.32}$$

The calculation procedure can be applied without approximation to systems of first order reactions. Application to Eqn. (3.49) which describes the relaxation of an isolated reaction yields immediately the relaxation time of this process.

It may be shown (Heinrich and Rapoport, 1975) that for a linear sequence of irreversible reactions the expression for $\hat{\tau}_k$ of a metabolite $S_k$ is simply the sum of the relaxation times of the individual reactions

$$\hat{\tau}_k = \sum_{i=j}^{k} \tau_i \quad (k \geq j) \tag{4.33}$$

where the index $j$ indicates the site of the initial perturbation. Consequently, the slowest enzyme will have the greatest transient control. The theoretical formulas are in accord with the experiments of Wurster and Hess (1970) and Barwell and Hess (1970). Figure 19 shows the time dependencies of the metabolites of a linear enzymatic chain with four components and the transition times calculated by formula (4.26).

The transition time may also be used for real nonlinear systems by replacing the integrals by sums and calculating the $\hat{\tau}_i$ during the integration. The upper limits of

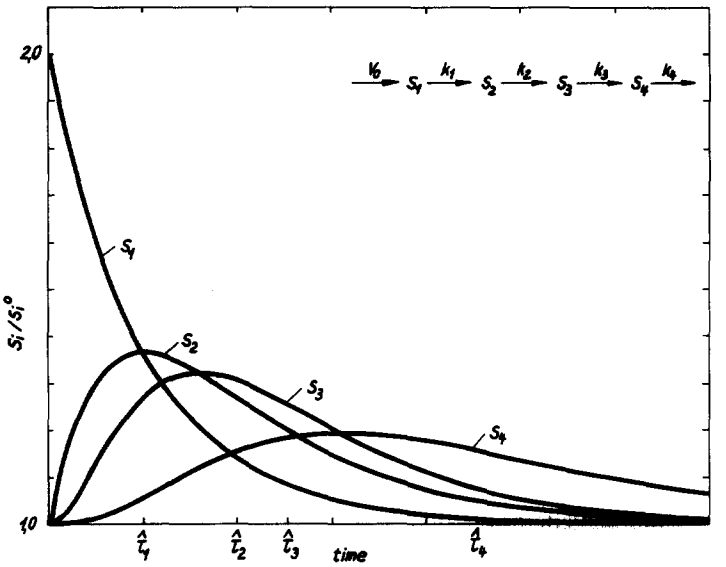



FIG. 19. Time dependencies of the concentrations and transition times $\hat{\tau}_i$ of the metabolites $S_i$ of a linear enzymatic chain after perturbation of the steady state. At $t = 0$ all metabolites were in the steady state ($S_i = S_i^0$) except the first metabolite which was raised two-fold. Parameter values: $v_0 = 1$, $k_1 = 1$, $k_2 = 1$, $k_3 = 2$, $k_4 = 0.5$.

the integrals can be chosen so that $\sigma_i/S_i^0$ is less than the experimental error of the determination of the metabolite $S_i$ (Rapoport *et al.*, 1976a).

The time of relaxation of the whole system may be quite different from that of the slowest enzyme. As an example we consider the linear system depicted in Scheme 13 which obeys the following differential equations

$$\frac{dS_1}{dt} = v_0 - k_1 S_1 - k_2 S_1 + k_{-2} S_2 \tag{4.34a}$$

$$\frac{dS_2}{dt} = k_2 S_1 - k_{-2} S_2 . \tag{4.34b}$$

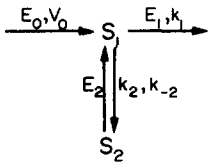
Assuming $k_2, k_{-2} \gg k_1$ one gets by application of the steady state approximation (Section III.5(b))

$$\frac{dS_1}{dt} = \frac{v_0}{1 + q_2} - \frac{k_1 S_1}{1 + q_2}, \quad q_2 = \frac{k_2}{k_{-2}}. \tag{4.35}$$

It is seen that the relaxation time of this process

$$\hat{\tau} = \frac{1 + q_2}{k_1} \tag{4.36}$$

is strongly influenced by the equilibrium constant of the fast reaction and may be much greater than $1/k_1$, the relaxation time of the slowest enzyme, if $q \gg 1$. The biological significance of Scheme 13 may be discussed for the synthesis and breakdown of cAMP (Newsholme and Start, 1973). $S_1$ may stand for cAMP, $E_0$ for the adenylate cyclase, $E_1$ for the diesterase and $S_2$ may be the complex of protein kinase and cAMP.




Scheme 13.

Reaction 2 is a non-enzymatical binding reaction. From the considerations given above it follows that the binding of cAMP to the protein kinase should not be too strong since otherwise its relaxation would be very slow and the cell could not respond to fast changes in the hormonal environment. Weak binding on the other hand means that only a small amount of enzyme can be activated and this is probably one reason for the existence of a cascade of activations in glycogen breakdown.

The transient control has to be distinguished clearly from the control parameters for the steady state. Enzymes which have a great influence on the transition time may be unimportant for the flux control in the steady state and vice versa. This statement is exemplified by Scheme 13, considered above. The steady state flux is determined by the enzyme $E_0$ alone whereas the transition control is exerted exclusively by the enzyme $E_1$ (under steady state approximation). Thus the relaxation of a metabolite depends primarily on the succeeding enzyme while the rate of formation of the metabolite has no influence.

## V. MODELS OF THE GLYCOLYTIC SYSTEM

The methods and general considerations presented in the preceding sections will be applied to a real metabolic system in this section.

The glycolytic system is by far the best known metabolic pathway. Many enzymes from various sources have been purified and characterized kinetically and there exist reliable data on all metabolite concentrations. In many cells under usual conditions the glycolytic pathway interacts with other pathways such as respiration, gluconeogenesis and the oxidative pentose pathway. Thus, its regularities are obscured and the attempts to apply simple models are foiled by their inadequacy. For such reasons the choice of appropriate simple biological systems is of great importance. Several biological systems possess nearly uncontaminated glycolytic pathway. First, mature mammalian erythrocytes the metabolism of which is reduced to glycolysis only with a minor contribution of the oxidative pentose pathway (Jacobasch *et al.*, 1974). They offer the additional advantage of the absence of organelles. Their enzymes and metabolites have been studied under a variety of conditions. Further systems are ascites tumor cells under anaerobic conditions (Schulz *et al.*, 1977) and anaerobic yeast cells.

One may also mention the eye lens, fetal liver and spermia which, however, have not been studied extensively.

Erythrocytes are probably the best object for the study of "pure" glycolysis. Any biological object has its particular properties and extensions to other systems are therefore not trivial. The main purpose of the section is to illustrate general statements and to show the regulatory principles of glycolysis. The fit of the model to the experimental data will not be considered in detail. Comparison with the data can be found in the original literature (Rapoport *et al.*, 1974; Rapoport *et al.*, 1976a) and is generally satisfactory.

### 1. *The Glycolysis of Erythrocytes*

#### (a) *Specification of the Model and Assumptions*

Figure 20 shows the scheme for the model of erythrocyte glycolysis. It includes the 2,3-bisphosphoglycerate bypass which is a characteristic feature of most mammalian erythrocytes. The oxidative pentose pathway has been disregarded whereas adenylate kinase and ATP-consuming processes were included. Processes which change the total amount of adenine or pyridine nucleotides were neglected because of their slowness (Lowy *et al.*, 1958). Conservation equations for these substances result. Non-equilibrium enzymes which are of regulatory importance include the hexokinase, phosphofructokinase, pyruvate-kinase, bisphosphoglycerate-mutase, 2,3-bisphosphoglycerate phosphatase and the non-glycolytic ATP-consuming processes (ATPase). For these enzymes simple rate laws were assumed (Table 5). All other enzymes represented in the model were assumed to be near to equilibrium. The most important interactions of metabolites

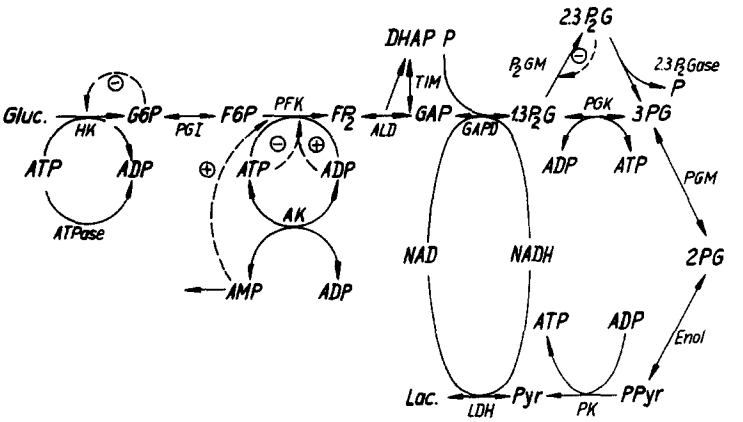



FIG. 20. Scheme for glycolysis in erythrocytes considered for the model. Arrows in both directions indicate reactions near to equilibrium. Arrows in one direction refer to practically irreversible reactions. The broken lines indicate activations (+) or inhibitions (−) of enzymes by metabolites, which were taken into account for the model.

with enzymes were included in the model: the inhibition of the hexokinase by glucose-6-phosphate, ATP-inhibition and AMP-activation of the phosphofructokinase and the inhibition of the bisphosphoglycerate-mutase by 2,3-bisphosphoglycerate. Reasons for the neglect of other interactions have been given (Rapoport *et al.*, 1974). In general, the rate laws and the parameter values of the enzymes correspond to those found for the isolated proteins *in vitro*. For the 2,3-bisphosphoglycerate phosphatase a rate law has been used which was established *in vivo*. *In vitro* data are difficult to apply owing to the complexity of the 2,3-bisphosphoglycerate-degradation (Harkness *et al.*, 1970; Rosa *et al.*, 1975; Sasaki *et al.*, 1975). For the ATPase a simplified *ad hoc* rate law was employed since the ATP-consuming processes are heterogeneous (Nakao, 1974) and an overall rate law has not been obtained so far. The hexokinase and phosphofructokinase have been lumped into one subsystem with a single response to the adenine nucleotides. An inherent assumption is that glucose 6-phosphate and fructose 6-phosphate are always in a quasi-steady state. It is justified by two considerations: first, glucose 6-phosphate and fructose 6-phosphate are near equilibrium owing to the rapidity of the hexose phosphate isomerase reaction, secondly by the low relaxation time of the phosphofructokinase (Table 6), which mainly determines the relaxation time of these metabolites. The net response of the hexokinase–phosphofructokinase-system to the ATP-concentration has been calculated from the kinetics of the individual enzymes (Fig. 21). The hexokinase displays an approximate Michaelis–Menten function with respect to ATP up to 2.0 mM (water basis). The phosphofructokinase is activated at low ATP-concentrations and inhibited allosterically at higher ones. Owing to the conservation equation a change of the ATP-concentration acts on the phosphofructokinase not

TABLE 5. RATE LAWS ASSUMED FOR THE VARIOUS ENZYMIC STEPS OF THE GLYCOLYTPC MODEL

| Enzyme | Rate law | Comments |
|---|---|---|
| hexokinase–phosphofructo–kinase system | $v_{HK-PFK} = \dfrac{V_{max}[ATP]/K_A}{1 + [ATP]/K_A + ([ATP]/K_i)}$ | The parameters in this rate law obtained by fitting to the net flux curve in Fig. 21 |
| pyruvate kinase | $v_{PK} = k_{PK}[ADP][P - Pyr]$ | |
| bisphospho glycerate mutase | $v_{P_2GM} = \dfrac{k_{P_2GM}[1,3\,P_2G]}{1 + [2,3\,P_2G]/K_{P_2G}}$ | |
| 2, 3-bisphospho glycerate phosphatase | $v_{P_2Gase} = \dfrac{k_{P_2Gase}[2,3\,P_2G]}{k_{P_2Gase} + [2,3\,P_2G]}$ | The rate law differs from that used previously (Rapoport *et al.*, 1976a). It is based on new experimental data (Rapoport *et al.*, 1976b) |
| ATPase | $v_{ATPase} = k_{ATPase}[ATP]$ | |

TABLE 6. RELAXATION TIMES OF THE NON-EQUILIBRIUM ENZYMES

| Enzyme | Relaxation time |
|---|---|
| hexokinase–phosphofructokinase system | 1.5 h |
| ATPase | 0.5 h |
| pyruvate kinase | 28 s |
| $P_2G$-bypass§ | 9.3 h |
| 2,3 bisphosphoglycerate mutase§ | 3.9 s |
| hexokinase† | 0.6 h |
| phosphofructokinase† | 74 s |

The relaxation times were calculated from the rate laws $v(S_i)$. $\tau$ is for a one substrate reaction $(\partial v/\partial S)^{-1}$ and for a two substrate reaction $[(\partial v/\partial S_1) + (\partial v/\partial S_2)]^{-1}$.

§ The relaxation time of the bypass was calculated by linearization of the differential equation for 2,3-bisphosphoglycerate (Eqn. (5.2)) with respect to 2,3-bisphosphoglycerate. For the 2,3-bisphosphoglycerate phosphatase no relaxation time can be calculated since this enzyme is practically saturated with its substrate.

† The relaxation times of the individual enzymes are shorter than those of the system owing to the glucose-6-phosphate inhibition of the hexokinase.

only by a variation of its inhibitory effect but also by virtue of the complementary changes of AMP. Fructose 6-phosphate has an activating influence on the phosphofructokinase. Both the hexokinase and phosphofructokinase curves were calculated from established kinetic models (Gerber *et al.*, 1974 and Otto *et al.*, 1974, respectively). The net flux through the hexokinase–phosphofructokinase system which is identical with the steady state glycolytic flux is given by the intersection of the individual curves. The net curve displays a maximum. Under *in vivo* conditions the flux is inhibited by an increase of the ATP-concentration.

A similar conclusion has been drawn recently by Ataullakhanov *et al.* (1977). They constructed the ATP-dependence of the glycolytic flux in erythrocytes by means of a computer and obtained qualitatively the same curves as shown in Fig. 21. Quantitative differences appear to be due to their assumption of a stronger glucose 6-phosphate inhibition of the hexokinase.

The dynamics of the glycolytic system is described by a system of ordinary differential equations. For every metabolite one equation can be written which takes into account its formation and degradation. Considering the time hierarchy (Section III.5) the system of differential equations can be reduced by eliminating the fast equilibrium reactions. The glycolytic system can thus be described by only four differential equations which have the following form:

$$\frac{d}{dt}(2[FP_2] + [TP] + [1.3P_2G] + [3PG] + [2PG] + [PPyr]) = 2v_{HK-PFK} - v_{P_2GM} + v_{P_2Gase} - v_{PK} \quad (5.1)$$

$$\frac{d[2.3P_2G]}{dt} = v_{P_2GM} - v_{P_2\,Gase} \quad (5.2)$$

$$\frac{d}{dt}(2[ATP] + [ADP] + 2[FP_2] + [TP] + [1.3P_2G]) = -v_{P_2GM} + v_{PK} - v_{ATPase} \quad (5.3)$$

$$\frac{d}{dt}([Lac] + [Pyr]) = v_{PK} - v_{exchange}. \quad (5.4)$$

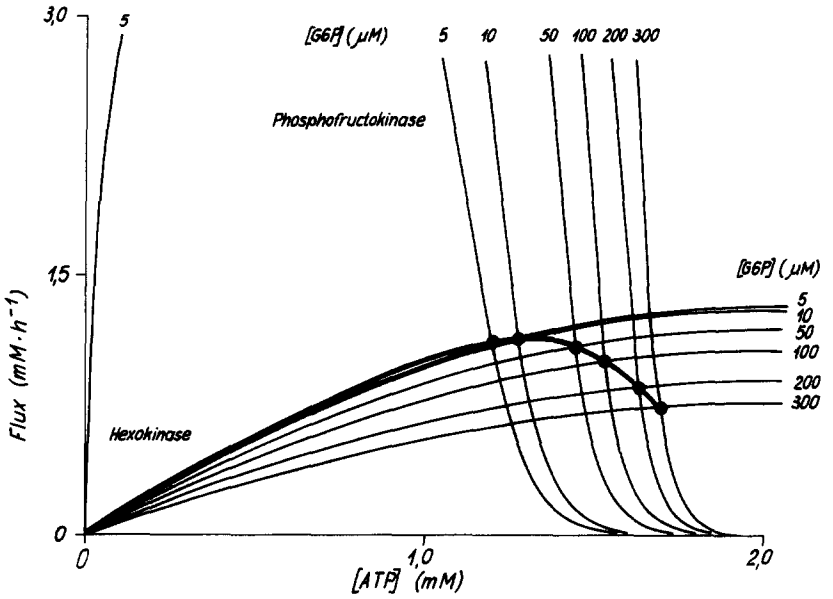



FIG. 21. Construction of the ATP-response of the hexokinase–phosphofructokinase system. The concentrations in the figure are given per litre of water. For the calculation of the hexokinase curves the kinetic model and the parameter values given by Gerber *et al.* (1974) were used. For the phosphofructokinase the following rate law was employed (see Otto *et al.* (1974)):

$$V_{PFK} = V_{max} \frac{\dfrac{0.4[G6P]}{K_F} \dfrac{[MgATP]}{K_{MgATP}}}{\left(1 + \dfrac{0.4[G6P]}{K_F}\right)\left(1 + \dfrac{[MgATP]}{K_{MgATP}}\right)} \frac{1}{1+L}$$

$$L = L_0 \frac{\left(1 + \dfrac{[ATP]_{free}}{K_{ATP}}\right)^4 \left(1 + \dfrac{[Mg]_{free}}{K_{Mg}}\right)^4}{\left(1 + \dfrac{0.4[G6P]}{K_F}\right)^4 \left(1 + \dfrac{[AMP]}{K_{AMP}}\right)^4}$$

The following parameter values were used (given per litre water) $V_{max} = 250\,\text{mM}h^{-1}$, $K_{MgATP} = 68\,\mu\text{M}$, $K_F = 100\,\mu\text{M}$, $K_{ATP} = 10\,\mu\text{M}$, $K_{Mg} = 440\,\mu\text{M}$, $K_{AMP} = 33\,\mu\text{M}$, $L_0 = 0.05 . \Sigma A$ and $Mg^{2+}_{total}$ were held fixed at 2.0mM. Only the complex formation of $Mg^{2+}$ and ATP was taken into account ($K_{ass} = 12.3\,\text{m}M^{-1}$). The concentration of AMP was calculated from the adenylate kinase equilibrium. The *in vivo*-point is characterized by the following concentrations of the metabolites which are given per litre of cells (experimental values in parenthesis, see also Gerber *et al.* (1973)): $[ATP]_{total} = 1.12\,\text{m}M$ (1.2), $[MgATP] = 0.99\,\text{m}M$ (1.05), $[ATP]_{free} = 0.13\,\text{m}M$(0.13), $[AMP] = 0.1\,\text{m}M$(0.05), $[G6P] = 73\,\mu\text{M}$(44), Flux $= 1.05\,\text{m}Mh^{-1}$ (1.1). The net response of the system was fitted with the equation given in Table 5. In the calculations with the whole glycolytic system ATP corresponds to $ATP_{total}$ of this detailed simulation.

$v_{exchange}$ denotes the exchange rate of pyruvate and lactate with other tissues of the body. Equation (5.3) is the result of three differential equations

$$\frac{d[ATP]}{dt} = -2v_{HK-PFK} + v_{PK} + v^+_{PGK} - v^-_{PGK} + v^+_{AK} - v^-_{AK} - v_{ATPase} \tag{5.5}$$

$$\frac{d[ADP]}{dt} = 2v_{HK-PHK} - v_{PK} - v^-_{PGK} + v^-_{PGK} - 2v^+_{AK} + 2v^-_{AK} + v_{ATPase} \tag{5.6}$$

$$\frac{d}{dt}([3PG] + [2PG] + [PPyr]) = v^+_{PGK} - v^-_{PGK} + v_{P_2Gase} - v_{PK} \tag{5.7}$$

where the + and − signs indicate the fast forward and backward reactions, respectively,

of the steps near to equilibrium. From these three differential equations a slow motion is extracted (see Section III.5(b))

$$\frac{d}{dt}(2[ATP] + [ADP] - [3PG] - [2PG] - [PPyr])$$

$$= -2v_{HK-PFK} + 2v_{PK} - v_{ATPase} - v_{P_2Gase}. \quad (5.8)$$

Finally, Eqn. (5.8) is added to Eqn. (5.1) yielding Eqn. (5.3). Analogously, Eqns. (5.1) and (5.4) were obtained by eliminating the fast reactions on the right side of the differential equations. There are four essential dynamic variables in the glycolytic system of glycolysis. (a) the pool of phosphorylated intermediates between the phosphofructokinase and pyruvatekinase (Eqn. (5.1)), (b) 2,3-bisphosphoglycerate (Eqn. (5.2)), (c) the pool of the energy rich phosphate compounds (Eqn. (5.3)) and (d) the pool of pyruvate and lactate. The essential dynamic variables are connected with the actual metabolites by equilibria and conservation relations. The following equilibria must be considered

$$q_{PGI} = \frac{[F6P]}{[G6P]}, \; q_{Ald} = \frac{[GAP]}{[FP_2]}, \; q_{TIM} = \frac{[GAP]}{[DHAP]}$$

$$[P_i]q_{GAPD} = q'_{GAPD} = \frac{[1.3P_2G][NADH]}{[GAP][NAD^+]}$$

$$q_{PGK} = \frac{[3PG][ATP]}{[1.3P_2G][ADP]}, \quad q_{PGM} = \frac{[2PG]}{[3PG]} \quad (5.9)$$

$$q_{Enol} = \frac{[PPyr]}{[2PG]}, \; q_{LDH} = \frac{[Lac][NAD^+]}{[Pyr][NADH]}, \quad q_{AK} = \frac{[ADP]^2}{[ATP][AMP]}.$$

On the basis of the conservation equation for the adenine nucleotides and the equilibrium relation for the adenylate kinase reaction the following equations can be obtained which express ADP and AMP as a function of ATP

$$[ADP] = \frac{q_{AK}[ATP]}{2}\left[\sqrt{1 + \frac{4}{q_{AK}}\left(\frac{A}{[ATP]} - 1\right)} - 1\right] \quad (5.10a)$$

$$[AMP] = A - [ATP] - [ADP]. \quad (5.10b)$$

By a transformation, the essential dynamic variables can now be expressed by four independent metabolites which may be used instead. Thus, the dynamics of only four metabolites characterizes the dynamics of the whole system and the time dependences of the other metabolites can easily be deduced. For methodical convenience the four independent metabolites recommended are ATP, 2,3-bisphosphoglycerate, 3-phosphoglycerate, and lactate. Of course, there may be substitutions, such as AMP or ADP for ATP, phosphoenolpyruvate or 2-phosphoglycerate for 3-phosphoglycerate and the glycolytic flux for lactate. In conditions where the hexokinase–phosphofructokinase system is not in a quasi-steady state glucose 6-phosphate or fructose 6-phosphate would have to be added as the fifth independent variable to characterize the interrelation between the two enzymes.

(b) *Properties of the Steady States*

In Fig. 22 are represented the steady state solutions of ATP and the fluxes versus the first order rate constant of the ATP-consuming processes. The points in the figure indicate the situation *in vivo*. Their position corresponds to the parameter set which gives the best agreement with the experimental data (see legend to Fig. 22). The dotted parts of the curves indicate unstable steady states. There is only a limited parameter range for which non-zero stationary solutions exist. If the rate constant $k_{ATPase}$ is increased too much only the trivial solution [ATP] = 0 remains. There is also a lower

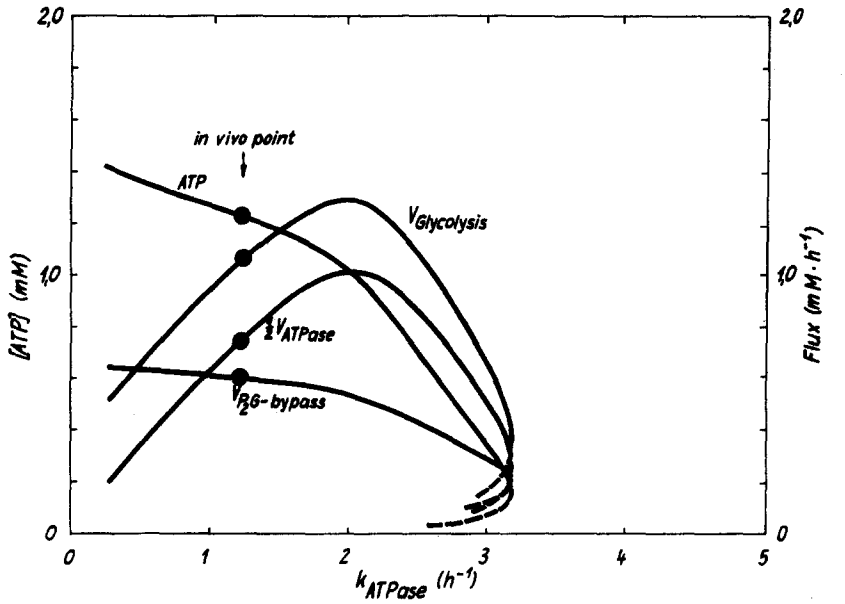



FIG. 22. ATP-concentration and fluxes in the steady state as functions of the rate constant of the ATPase ($k_{ATPase}$). The $v_i$ denote fluxes specified by the superscript. The curves were calculated with the following parameter values: $k_{P_2GM} = 1.1 \times 10^5\ h^{-1}$, $k_{P_2Gase} = 2.13\ h^{-1}$, $k_{PK} = 0.67\ h^{-1}$, $q_{Enol}\, q_{PGM}\, q_{PGK} = 300$, $K_{P_2G} = 40\ \mu M$, $K_{P_2Gase} = 300\ \mu M$, $V_{PFK} = 2.9\ mM\ h^{-1}$, $K_A = 1168\ \mu M$, $K_i = 1241\ \mu M$, $n = 12$, $\Sigma A = 1.4\ mM$. At the *in vivo*-point ($k_{ATPase} = 1.23\ h^{-1}$) the following concentrations of the metabolites were calculated (experimental values in parenthesis): [ATP] = 1.22 mM (1.2), [ADP] = 144 $\mu$M (185), [AMP] = 34 $\mu$M (50), [2,3 $P_2G$] = 4.49 mM (4.7), [P-Pyr] = 21 $\mu$M (26), [1,3 $P_2G$] = 0.58 $\mu$M (0.5), Flux = 1.05 mM $h^{-1}$ (1.1 mM $h^{-1}$). The dotted parts of the curves indicate unstable steady states.

limit for the $k_{ATPase}$-values. Two mechanisms, the first non-stoichiometric and the second stoichiometric, contribute to the relative constancy of ATP in the region of physiological interest. The first one is connected with the ATP-inhibition and AMP-activation of the phosphofructokinase. If $k_{ATPase}$ is increased, the concentration of AMP rises and that of ATP falls. This leads to an activation of the phosphofructokinase and, via the

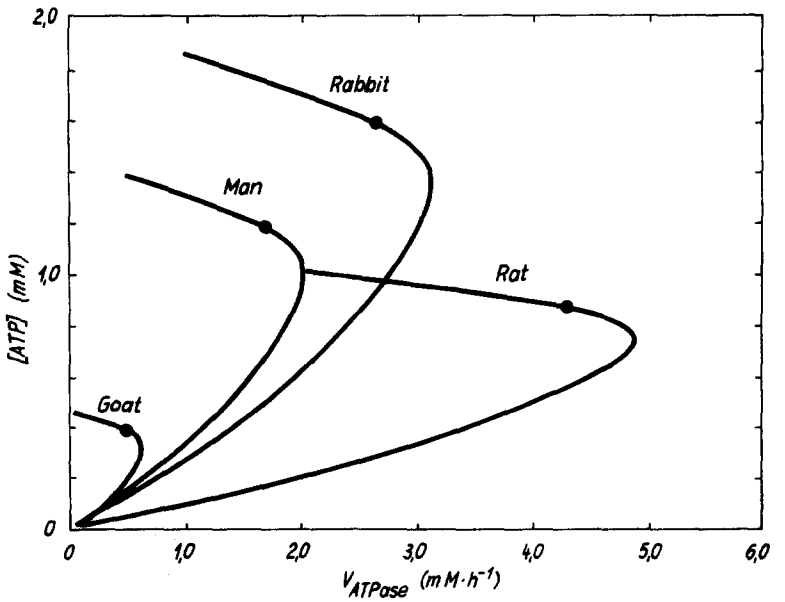



FIG. 23. ATP-concentration as a function of the rate of the non-glycolytic ATP-consuming processes ($v_{ATPase}$) for erythrocytes from various species. The parameter values for the enzymes of different species were taken from Jacobasch (1970) and Rapoport *et al.* (1976a). The points give the *in vivo*-points for the cells.

inhibition of the hexokinase by glucose 6-phosphate, to an increased glucose consumption (Fig. 22). The higher flux produces more ATP in the lower part of the glycolytic chain and so compensates for the increased rate of ATPase. The second mechanism is of lesser importance and is connected with the 2,3-bisphosphoglycerate-bypass. The increase of $k_{ATPase}$ leads also to a higher ADP-concentration and to a lowered concentration of 1,3-bisphosphoglycerate and a smaller rate of the bisphosphoglycerate-mutase (Fig. 22). The compensatory increase of the flux through the phosphoglycerate kinase leads to an additional ATP-production and again the ATP-level is stabilized.

Figure 23 gives plots of the ATP-concentration versus the rate of the ATP-consuming processes (not their rate constant) for the erythrocytes of various animal species. The curves display a maximum in the ATP-consumption rate.

The localization of the *in vivo* points is of interest (see Fig. 23). The points lie all near to the maxima of the curves, so that about 85% of the maximally possible work is performed. This represents probably an evolutionary adaptation. This is clearly different from cells which are geared for great changes in the ATP-need (e.g. muscle cells). Erythrocytes can, however, do more work during the quasi-steady state period at unrelaxed concentrations of 2,3-bisphosphoglycerate (see below) than in the steady state.

(c) *Time Dependent Processes; The Existence of Quasi-Steady States*

The glycolytic system is characterized by a pronounced time hierarchy of the reactions. Table 6 gives the calculated relaxation times of the non-equilibrium enzymes. They vary over almost four orders of magnitude. Accordingly, the eigenvalues of the system calculated at the *in vivo* point differ by more than two orders of magnitude (Rapoport *et al.*, 1976a). There is a fast variable, represented by phosphoenolpyruvate, an intermediate one (ATP) and a very slow one (2,3-bisphosphoglycerate). Figure 24 gives a phase plane plot of the concentration of ATP versus the concentration of 2,3-bisphosphoglycerate obtained by numerical integration of the differential equations for different starting values of the metabolites. There is initially a fast movement toward the quasi-steady state line (dotted curve). This takes about 0.5–2 h. The quasi-steady state line is determined by the condition that the time derivatives in Eqns. (5.1) and (5.3) are zero. Having approached the line the system moves slowly toward the stationary *in vivo* point. The slow movement is determined by the relaxation of 2,3-bisphosphoglycerate and takes more than 10 h. The first phase is characterized by an almost constant concentration

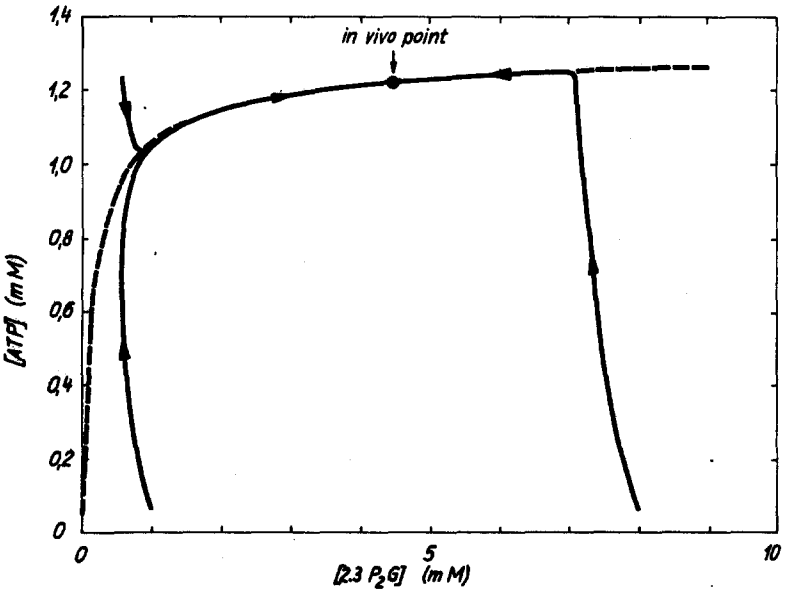



FIG. 24. Relaxation of ATP and 2,3-bisphosphoglycerate to the steady state. The continuous lines give the trajectories of the movements from several starting points. The steady state which is finally approached is indicated. The broken line indicates the quasi-steady state.

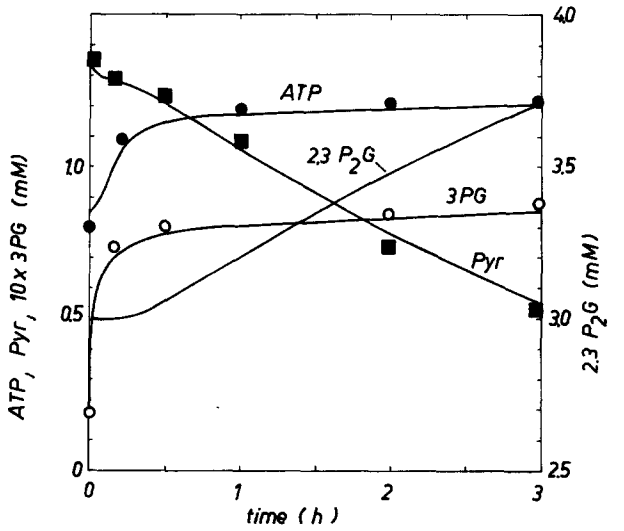



FIG. 25. Experimental proof for the existence of a quasi-steady state at unrelaxed 2,3 bisphosphoglycerate concentrations. Erythrocytes were preincubated for 2 h at pH 7.6 in the absence of glucose. Then glucose was added and the time-dependent changes of the metabolite concentrations were measured. The points in the figure represent experimental values (Tomoda and Minakami, 1975), the curves were calculated (Rapoport *et al.*, 1976a). Beyond 0.5 h a quasi-steady state period is observed for ATP and 3-phosphoglycerate, but 2,3-bisphosphoglycerate and consequently pyruvate change continuously.

of 2,3-bisphosphoglycerate, in the second phase the concentration of 2,3-bisphosphoglycerate changes greatly while ATP and all other metabolites remain nearly constant. The quasi-steady state might appear to the experimenter as a true steady state since the changes of 2,3-bisphosphoglycerate occur only slowly.

In Fig. 25, experimental proof for the existence of a quasi-steady state is given. The points in the figure represent experimental data (Tomoda and Minakami, 1975), the curves were calculated.

Figure 26 shows the effect of $k_{ATPase}$ on the ATP-level in the quasi-steady state. For comparison the line of the true steady state is included in the figure. ATP is more

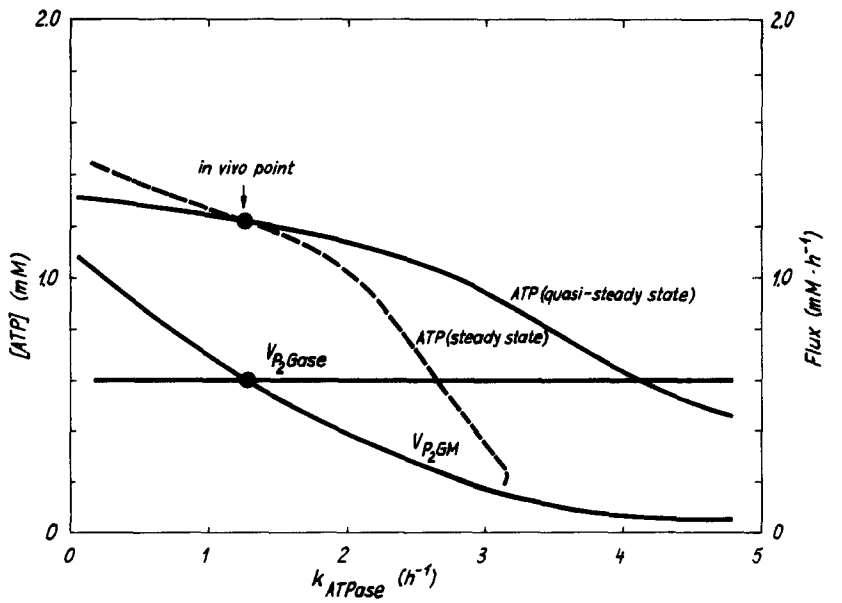



FIG. 26. ATP-concentration and fluxes through the bypass in the quasi-steady state as functions of the rate constant of the ATPase ($k_{ATPase}$). The continuous lines are those for the quasi-steady state at unrelaxed 2,3-bisphosphoglycerate concentrations, the broken line corresponds to the true steady state (cf. Fig. 22). The curves were computed with [2,3 $P_2G$] = 4.49 mM. At the *in vivo*-point $v_{P_2GM} = v_{P_2Gase}$ and $[ATP]_{steady\ state} = [ATP]_{quasi\text{-}steady\ state}$.

constant in the quasi-steady state. This means that the glycolytic system can tolerate ATP-overconsumption for a short period of time better than for longer periods. The reason for this additional constancy of ATP is that an increased $k_{ATPase}$-value leads to a lowered rate of bisphosphoglycerate-mutase while the 2,3-bisphosphoglycerate phosphatase is not affected initially. Therefore more 2,3-bisphosphoglycerate is degraded than formed during the quasi-steady state period and this additional flux yields ATP at the pyruvate kinase step. Thus 2,3-bisphosphoglycerate acts as an energy source for a certain period of time. The role of 2,3-bisphosphoglycerate as an energy source is employed for blood preservation where ATP is buffered at the expense of 2,3-bisphosphoglycerate-degradation (Rapoport, 1947). Only the steady state with [ATP] = zero exists, presumably because the $k_{ATPase}$-value is increased too strongly relative to the $V_{max}$ of the hexokinase–phosphofructokinase system.

(d) *The Role of Conservation Quantities* in vitro

Conservation quantities for the adenine and pyridine nucleotides, phosphate groups and for the oxidation equivalents exist *in vitro*. The following equations may be written considering only the glycolytic pathway

$$\text{adenine nucleotides} = [\text{AMP}] + [\text{ADP}] + [\text{ATP}] \tag{5.11}$$

$$\text{pyridine nucleotides} = [\text{NAD}^+] + [\text{NADH}] \tag{5.12}$$

$$\begin{aligned}\text{phosphate groups} = {} & [\text{G6P}] + [\text{F6P}] + [2\,\text{FP}_2] + [\text{TP}] + 2[1,3\,\text{P}_2\text{G}] + [3\,\text{PG}] \\ & + [2\,\text{PG}] + [\text{PPyr}] + 2[2,3\,\text{P}_2\text{G}] + [\text{ADP}] + 2[\text{ATP}] \\ & + [P_i]\end{aligned} \tag{5.13}$$

$$\begin{aligned}\text{oxidation equivalents} = {} & [\text{NAD}^+] + [1,3\,\text{P}_2\text{G}] + [3\,\text{PG}] + [2\,\text{PG}] + [\text{PPyr}] \\ & + [\text{Pyruvate}] + [2,3\,\text{P}_2\text{G}].\end{aligned} \tag{5.14}$$

For the first two quantities the conservation restriction may also hold *in vivo* for certain periods of time since the processes changing these sums are very slow. For phosphate groups the approximation is valid only for a short time since inorganic phosphate is transported through the cell membrane with a half time of 1–2 h. For the oxidation equivalents no conservation equation is valid *in vivo* since pyruvate is exchanged rapidly with other tissues. The conservation equation for the oxidation equivalents *in vitro* has been often overlooked. The constancy of the sum of oxidized metabolites has recently been tested experimentally (Rapoport *et al.*, 1976b). Generally, the expected inverse relation between 2,3-bisphosphoglycerate and pyruvate-changes has been found. In some conditions there exist quantitative discrepancies indicating that additional oxidation or reduction equivalents become available. Oxidation equivalents may be introduced into the system at the glyceraldehydephosphate dehydrogenase step via the methemoglobin reductase (Travis *et al.*, 1974). The source of the reduction equivalents is unknown.

Figure 27 shows the theoretical curves for the accumulation of 2,3-bisphosphoglycerate at different amounts of oxidation equivalents. The conservation sum limits the changes of 2,3-bisphosphoglycerate and influences the transition time of the system. The final state of the system *in vitro* is characterized by quasi-stationary concentrations of all metabolites except for the accumulation of lactate, fructose 1,6-bisphosphate and triose phosphate. The increase of the phosphate esters is due to the constancy of pyruvate and consequent continuous rise of NADH. The smaller the conservation quantity the greater the effects in the system. Table 7 lists the calculated quasi-stationary concentrations of the metabolites *in vitro*. The production of fructose 1,6-bisphosphate and triose phosphate consumes ATP and results in a decreased ATP-concentration. An activation of the hexokinase–phosphofructokinase system may therefore result in a lowered ATP-level despite a higher glucose consumption rate. The quasi-steady state *in vitro* is due to the increase of [NADH] and the resulting decrease of $[\text{NAD}^+]$. Since $[\text{NAD}^+] \gg [\text{NADH}]$ such changes have little effect on the metabolite concentrations.

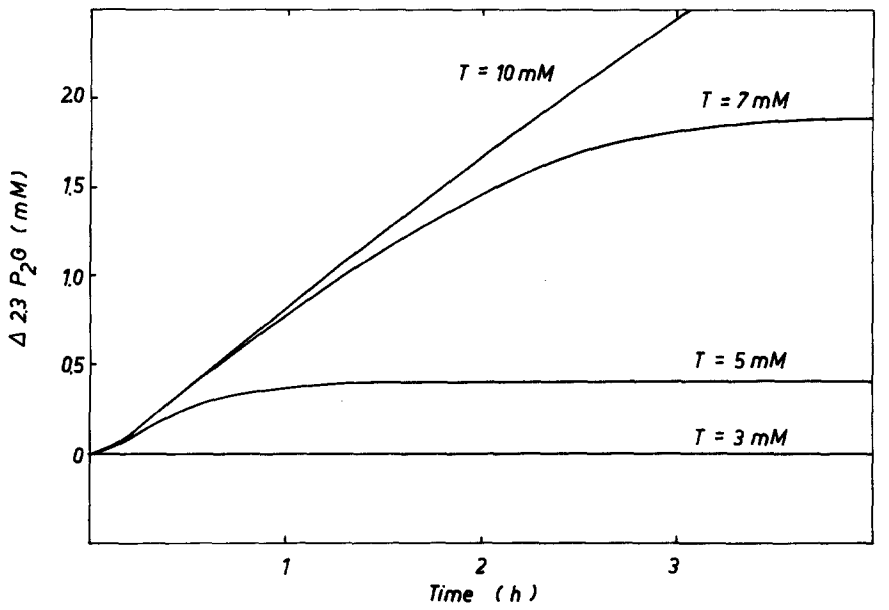



FIG. 27. Relaxation of 2,3-bisphosphoglycerate after a twofold activation of the hexokinase–phosphofructokinase system at different concentrations of the oxidation equivalents. $\Delta$[2,3 $P_2G$] is defined as [2,3 $P_2G$] ($t$) − [2,3 $P_2G$] ($t$ = 0). The starting values for 2,3-bisphosphoglycerate depend on the conservation sum $T$. The transition times were calculated by use of expression (4.26) at different levels of the oxidation equivalents. For $T$ = 10, 7 and 5 mM the values were 1.29, 0.66 and 0.21 h respectively. No relaxation could be observed for the lowest conservation sum $T$ = 3 mM, since 2,3-bisphosphoglycerate changes by less than 2%.

The analysis shows that the energy metabolism is the "core" of glycolysis. Thus, the status of the adenine nucleotides determines the redox state of the pyridine nucleotides and not conversely. This conclusion is in agreement with that drawn by Stubbs *et al.* (1972) in an intuitive manner.

The conservation of the phosphate groups has been investigated by Glende *et al.* (1975) for a simplified glycolytic system. Their model and the differential equations used are shown in Fig. 28 and the corresponding legend. In Fig. 29 the steady state curve of ATP versus the rate constant for the ATPases is given (compare with Fig. 22). The limitation by phosphate groups leads to a decreased ATP-level. In contrast to the unconstrained system activation of the ATPase at low $k_{ATPase}$-value results in an increased ATP-level with higher glycolytic flux. It follows that in erythrocytes phosphate limitation may result in lowered ATP and 2,3-bisphosphoglycerate levels.

A change in the conservation sum for the adenine nucleotides is considered in Figs. 30 and 31. The net response of the hexokinase–phosphofructokinase system (Fig. 30) was constructed as in Fig. 21. An increase of the conservation sum does not change

TABLE 7. DEPENDENCE OF THE QUASI-STEADY STATE CONCENTRATIONS *in vitro* ON THE CONSERVATION SUM FOR THE OXIDIZED METABOLITES (FROM RAPOPORT *et al.*, 1976a)

| | Conservation sum $T$ | | | | | |
|---|---|---|---|---|---|---|
| | $T = \infty$ | | $T$ = 5000 | | $T$ = 4500 | |
| Metabolite | control | activated | control | activated | control | activated |
| ATP | 1186 | 1354 | 1162 | 1133 | 1149 | 1103 |
| 2,3 bisphosphoglycerate | 4900 | 29470 | 4438 | 4872 | 4183 | 4390 |
| phosphoenolpyruvate | 263 | 215.6 | 23.8 | 32.2 | 22.4 | 29.1 |
| pyruvate | ∞ | ∞ | 482 | 19.6 | 254 | 15.4 |
| $\Delta$ flux | 0 | 0 | 33.2 | 1436 | 57.2 | 1557 |

The hexokinase–phosphofructokinase system was activated by a factor of two. $\Delta$ flux is the difference between the rates of glucose consumption and lactate formation given in triose units ($\mu$M $h^{-1}$). The concentrations are given in $\mu$M.

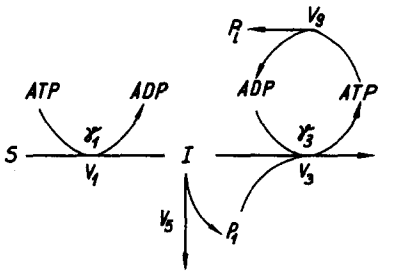



FIG. 28. Schematic representation of the phosphate metabolism in glycolysis. In the reaction 1 there are consumed $\gamma_1$ molecules ATP. $S$ stands for glucose and is considered to be constant. In the reaction $3\gamma_3$ molecules ATP are produced. $I$ stands for an energy rich intermediate (e.g. the triose phosphates). There is also a side-path (reaction 5) which can be either a leakage of energy rich compounds (e.g. 2.3 $P_2G$-bypass) or a biosynthetic pathway. In the reaction 9 ATP is hydrolyzed to ADP and $P_i$. The following equations are used for the description of the system:

$$\frac{d[I]}{dt} = k_1[\text{ATP}] - k_5[I] - k_3[I][\text{ADP}][P_i]$$

$$\frac{d[\text{ATP}]}{dt} = -k_1\gamma_1[\text{ATP}] + k_3\gamma_3[I][\text{ADP}][P_i] - k_9[\text{ATP}]$$

$$[P_0] = [\text{ATP}] + \gamma_1[I] + [P_i]$$

$$\Sigma A = [\text{ATP}] + [\text{ADP}].$$

significantly the maximal flux through the glycolytic chain. This is due to the fact that simultaneous increases of ATP and AMP compensate each other in their effects on the phosphofructokinase. Based on the curves in Fig. 30 the glycolytic model responded as shown in Fig. 31 to a change in the sum of adenine nucleotides. The ATP-concentration increased to approximately the same extent as the total sum so that the relations between the adenine nucleotides remained fairly constant. The glycolytic flux was only changed by 15% and the flux through the bypass was practically unchanged. These results are in agreement with experimental data (Syllm-Rapoport *et al.*, 1962, 1969) although the model still neglected important factors such as variations in the interactions of ATP, 2,3-bisphosphoglycerate, $Mg^{2+}$ and hemoglobin. The model shows that the

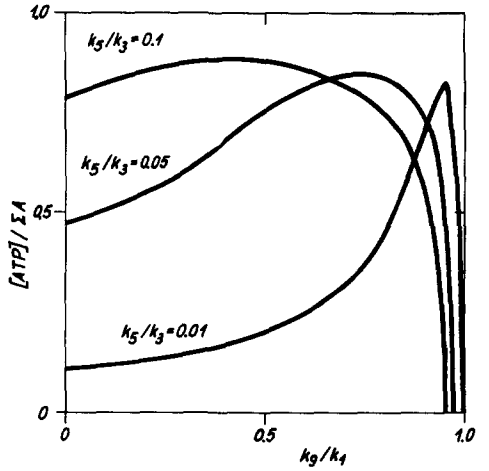



FIG. 29. Dependence of the ATP-concentration in the steady state on the rate constant of the ATPase under phosphate limitation (from Glende *et al.* (1975)). The curves were calculated on the basis of the reaction scheme given in Fig. 28. The ATP-concentration has been normalized to $\Sigma A$, the $k_{\text{ATPase}} = k_9$ to $k_1$ (rate constant of the "sparking" reaction). The parameters used were: $\gamma_3 = 2$, $\gamma_1 = 1$, $\Sigma$ phosphate/$\Sigma A = 5.0$ ($\Sigma$ phosphate is the conservation sum $P_0$ for phosphate groups). The ratio $k_5/k_3$ stands for the efficiency of the side-path. Two "advantageous" features of the model are apparent: 1, the ATP level rises if the load is increased ("compensation" effect); 2, if the side pathway is activated the ATP concentration increases.

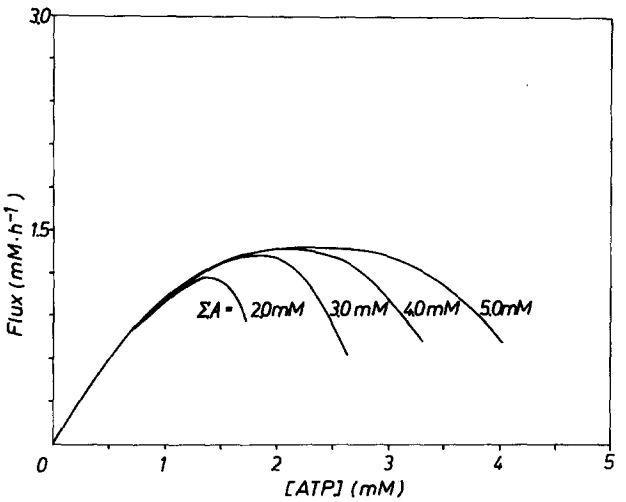



FIG. 30. ATP-response of the hexokinase–phosphofructokinase system at different total concentrations of the adenine nucleotides. The curves were constructed as described in the legend to Fig. 21 for different values of $\Sigma A$.

particular dependence of the velocity of the phosphofructokinase on the concentrations of ATP and AMP serves to keep constant the erythrocyte metabolism even in the case of great variations in the individual concentrations.

The conservation sum for the pyridine nucleotides has only a negligible influence on the system (Rapoport *et al.*, 1976a). Even a severalfold increase of the pyridine nucleotide pool should have no influence on either quasi-steady state levels *in vitro* or on the relaxation time of the system. The conservation sum of pyridine nucleotides should also have no influence *in vivo*.

(e) *Regulatory Enzymes of the Glycolysis of Erythrocytes*

The regulatory importance of the enzymes has been evaluated for steady states, quasi-steady states and time-dependent processes.

The control strengths are given in Table 8 of the glycolytic non-equilibrium enzymes in the steady state and in the quasi-steady state at unrelaxed concentrations of 2,3-bis-

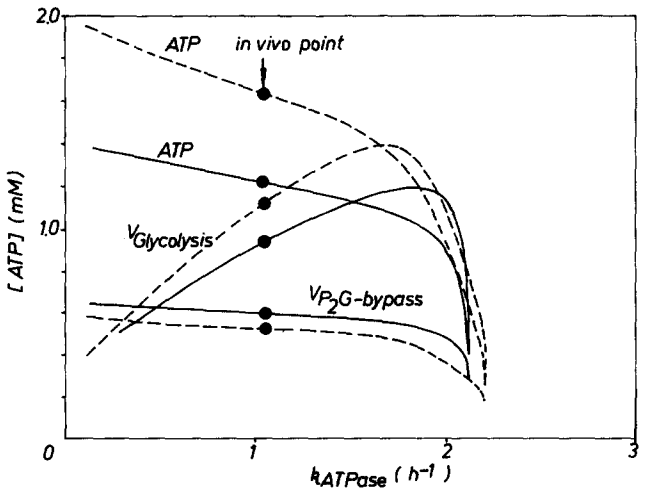



FIG. 31. Concentration of ATP and fluxes in steady states at different total adenine nucleotides. The continuous lines were calculated for $\Sigma A$ = 1.4 mM, the broken ones for $\Sigma A$ = 2.1 mM (per litre cells). The net curves of the hexokinase–phosphofructokinase system (Fig. 30) were employed. The curve at 1.4 mM does not entirely coincide with that given in Fig. 26 since the net curves of Fig. 30 were fitted with different parameter values to simulate the dependence on $\Sigma A$.

TABLE 8. CONTROL STRENGTHS OF THE GLYCOLYTIC NON-EQUILIBRIUM ENZYMES IN THE STEADY STATE AND IN THE QUASI-STEADY STATE AT UNRELAXED CONCENTRATIONS OF 2,3-BISPHOSPHOGLYCERATE

| Enzyme | Steady state | Quasi-steady state |
|---|---|---|
| hexokinase–phosphofructokinase system | 0.24 | 0.78 |
| ATPase | 0.55 | 0.25 |
| pyruvate kinase | −0.02 | −0.16 |
| bisphosphoglycerate mutase | 0.02 | 0.17 |
| 2,3-bisphosphoglycerate phosphatase | 0.21 | −0.04 |

phosphoglycerate. For steady state conditions the flux control is exerted by the hexokinase–phosphofructokinase-system, the ATP-consuming processes and by the 2,3-bisphosphoglycerate phosphatase. The other non-equilibrium enzymes and, of course, the equilibrium enzymes, do not influence the glycolytic flux. In the quasi-steady state the hexokinase–phosphofructokinase system has the most pronounced influence on the flux. This is the expected behaviour for a linear enzymatic chain if no strong feedback signals to the first enzymes exist (see Section IV.2.(d)). The nonequilibrium enzymes affect the metabolite concentrations differently. For instance, the pyruvate kinase which is rather unimportant for the flux control has an influence on the concentration of phosphoenolpyruvate in the steady state and in the quasi-steady state (Rapoport *et al.*, 1976a). The ATP-level is almost uninfluenced by all enzymes in the quasi-steady state at unrelaxed concentrations of 2,3-bisphosphoglycerate (cf. Fig. 26). Whereas the importance of the hexokinase–phosphofructokinase-system has been accepted by all experimenters additional flux controls have been falsely ascribed to the glyceraldehydephosphate dehydrogenase, phosphoglycerate kinase and pyruvate kinase (e.g. Reinauer and Bruns, 1964; Minakami, 1968). The time dependent processes are largely determined by the very slow relaxation of 2,3-bisphosphoglycerate as a result of the time limitation by the 2,3-bisphosphoglycerate phosphatase and bisphosphoglycerate mutase. The initial process toward the quasi-steady state is influenced more or less by all non-equilibrium enzymes of the system. The actual contribution of the various enzymes to the control of the transition time is, of course, dependent on the kind of perturbation of the system.

### 2. *Extensions to Other Cells*

Owing to the simplicity of the erythrocyte metabolism, most extensions to other types of cells involve the inclusion of additional, complicating features. These include the interaction with other pathways. In most cells respiration interacts with glycolysis. One result is the absence of a conservation quantity for oxidized glycolytic metabolites. Oxidation of NADH via the respiratory chain introduces oxidation equivalents into the glycolytic chain. The occurrence of respiration is linked to the presence of mitochondria which constitute a separate compartment. Of the various substances compartmentalized the most important ones are the adenine nucleotides. They contain much higher concentrations of ATP and ADP and in a ratio which differs from that in the cytosol. For some cells, e.g. for reticulocytes, the suppression of glycolysis by respiration (Pasteur effect) is partly due to the low cytosolic concentrations of the adenine nucleotides (Jacobasch *et al.*, 1975). The presence of a sizable oxidative pentose pathway requires its consideration in the model. This applies even to erythrocytes at low pH-values (Loecker, 1964). The occurrence of gluconeogenesis brings with it the consideration of its enzymes. Of necessity additional controlling steps will have to be introduced. There is good evidence that some futile cycling occurs in the liver (Katz and Rognstad, 1976) and to a large extent in the flying muscle of the bumble bee (Clark *et al.*, 1973; Bloxham *et al.*, 1973). Of course, if the first substrate is not glucose (e.g. galactose, pentoses,

glycogen) corresponding modifications of the model have to be introduced. In the presence of additional pathways other feedback signals may assume importance, e.g. the influence of alanine on the pyruvate kinase (Sparmann *et al.*, 1973). Obviously, only the kinetic constants of such enzymes which are germane to the cell type considered should be employed. For instance, the properties of the phosphofructokinase of erythrocyte and of muscle differ somewhat and there are large distinctions of both of them as compared with the phosphofructokinase of yeast.

A much disputed question is the functional importance of membrane binding of enzymes. The first precondition for such an effect to be of significance is that the enzyme exerts an influence on flux or metabolites. For the hexokinase of rabbit reticulocytes it appears likely that the binding may play a role in the Pasteur effect (Gellerich and Augustin, 1975). In other tissues, e.g. brain, binding occurs (Knull *et al.*, 1974) but its functional significance is uncertain. The observed binding of large amounts of glyceraldehydephosphate dehydrogenase (Schrier, 1963) and of minor amounts of other equilibrium enzymes on erythrocyte membranes lacks on two counts biological relevance (Maretzki *et al.*, 1974). First, because the enzymes do not exert control on glycolysis and secondly, because of the artefactual nature of the binding; it occurs only in hypotonic conditions.

The simple model for erythrocytes (Rapoport *et al.*, 1974) has been applied to two systems. In anaerobic ascites tumor cells (Schulz *et al.*, 1976) only the following modifications were necessary. First, the presence of an active $\alpha$-glycerophosphate-dehydrogenase had to be taken into account, secondly, the 2,3-bisphosphoglycerate-bypass could be dispensed with and thirdly, a unidirectionality and inhibition by NADH was assumed for the glyceraldehydephosphate dehydrogenase. The formation of $\alpha$-glycerophosphate leads to a modified conservation equation

$$[NAD^+] + [1.3P_2G] + [3PG] + [2PG] + [PPyr] + [pyr] - [\alpha GP] = \text{const.} \quad (5.15)$$

It follows from it that the accumulation of $\alpha$-glycerophosphate is accompanied by a stoichiometric formation of pyruvate in the steady state of the other metabolites of glycolysis. These predictions correspond to the experimental data.

The fetal rat liver which has been studied by Berger, R. and Hommes, F. A. (personal communication) is characterized by the absence of gluconeogenesis and glucose consumption, and a negligible respiration. In the metabolic sequence phosphorylase is the first enzyme. It is inhibited by uridindiphosphate-glucose. Under the assumption that this compound is in equilibrium with glucose 1-phosphate and glucose 6-phosphate the model becomes virtually identical with that of the glycolysis of the erythrocyte. Thus it has been shown that phosphorylase and, to a lesser extent, phosphofructokinase determine the steady state flux.

### 3. *Other Models of Glycolysis*

#### (a) *Detailed Models*

These models are characterized by the endeavour to include in a detailed manner all reactions with as many of their parameters as possible.

The pioneering work has been done by Chance *et al.* (1960) and Garfinkel and Hess (1964) on the aerobic glycolysis in ascites tumor cells. The latter model included eighty-nine reactions among sixty-five chemicals (enzymes, substrates, complexes etc.) and described the observed initial and final states quantitatively and the transitory behaviour of the system semi-quantitatively. Oxidative phosphorylation and compartmentation of ATP and $NAD^+$ were included in the model. Several *ad hoc* assumptions were necessary to obtain a satisfactory fit to the data. One served to circumvent the observed discrepancy in the aldolase–triose–phosphate–isomerase region where the mass action ratio was not in accord with the direction of the glycolytic flux. An interaction between the enzymes influenced by dihydroxyacetone phosphate was postulated but could not

be supported by later experiments (Hess, B., personal communication; Melnick and Holtin, 1973). Essential biological conclusions of the early work were: 1. There is general agreement of the kinetics of enzymes *in vitro* and *in vivo* despite some discrepancies. Thus, the working of a living cell may be simulated on the basis of known physico–chemical laws. 2. The adenine nucleotides are the most important factor for control of the glycolysis. 3. There is no single control site as in some biosynthetic pathways. Instead, the multitude of interactions *in vitro* between enzymes and effectors seemed to be important. 4. The equilibrium relation at the lactate dehydrogenase and glyceraldehyde-phosphate dehydrogenase postulated by Hohorst *et al.* (1959) could not be verified in the simulations. Instead, neither enzyme seemed to be at equilibrium and the glyceraldehyde phosphate dehydrogenase had a pronounced influence on the $NAD^+/NADH$-ratio. 5. The α-glycerophosphate dehydrogenase seemed to be of little importance in aerobic ascites tumor cells.

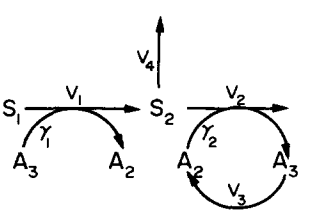
Later on Garfinkel *et al.* (1968) modified the model to describe the glycolysis of a beef heart supernatant. Again, simplifying assumptions on the enzyme mechanisms and many alterations of the *in vitro* data were necessary. The glycolytic flux became inhibited after a short time of incubation which seemed to be the result of a "coherent" regulation of the pathway as a whole, rather than of an inhibition of a single step. A discrepant behaviour of the triosephosphate isomerase–aldolase region was again noted and seems to be of general occurrence. The abnormality could be due to the hydration of glyceraldehyde 3-phosphate, to the anomerization of fructose 1,6-bisphosphate (Hess, B., personal communication) or to the binding of glyceraldehyde 3-phosphate to the enzymes which are present in high concentrations.

Vergonet (1971) employed a similar approach to the liver of fetal and adult rats. The model was designed to answer the question why fetal liver tolerates anaerobiosis better than adult liver. It could be shown that the glycolytic flux is mainly controlled by the hexokinase while the phosphofructokinase exerts only a fine control. The flux was roughly proportional to the concentration of ATP. While the energy production in fetal liver relied solely on glycolysis, in adults the transition to anaerobiosis leads to glycogen degradation which partly compensates for the decrease in energy production (Hommes *et al.*, 1973).

Detailed models of glycolysis have been built up also for the simulation of the oscillatory behaviour of the intermediates (Achs and Garfinkel, 1968; Richter *et al.*, 1975; see Section V.4).

(b) *Stoichiometric Models*

Selkov (1975a) proposed a skeleton model of glycolysis which takes into account only the stoichiometric structure of the pathway. The model is depicted in Scheme 14. The metabolite $S_1$ stands for glucose, $S_2$ for energy rich metabolites of the middle section of the glycolytic chain, $A_2$ and $A_3$ represent the adenine nucleotides ADP and ATP. The system consists of the following reaction steps: 1. The ATP-consuming processes of the upper part of glycolysis (hexokinase, phosphofructokinase, $v_1$); 2. the ATP-producing reactions of the lower part of glycolysis (phosphoglycerate kinase, pyruvate kinase, $v_2$); 3. non-glycolytic ATP-consuming processes, ATPases, $v_3$); 4. irreversible utilization of energy rich intermediates for synthesis ("leakage", $v_4$). The concentration of the first metabolite $S_1$ is assumed to be constant. The pathway is characterized




Scheme 14.

by reflexive catalysis (Calvin, 1969), i.e. a special type of autocatalysis where the product ATP is partly used to activate the initial substrate $S_1$ in a "sparking reaction". $\gamma_1$ and $\gamma_2$ are stoichiometric coefficients which indicate how many molecules of ATP are consumed in reaction $v_1$ and are produced in reaction $v_2$, respectively. It is assumed that the velocities of the enzymes depend linearly on the metabolite concentrations.

For the steady state concentrations of ATP the following equation is obtained

$$\frac{A_3}{A} = 1 - \frac{\left(\gamma_1 + \dfrac{k_3}{k_1 S_1}\right)\dfrac{k_4}{k_2 A}}{(\gamma_2 - \gamma_1) - \dfrac{k_3}{k_1 S_1}}. \tag{5.16}$$

$A$ denotes the total concentration of adenine nucleotides and the $k$'s are the rate constants. There exists a further steady state for which the concentrations of $S_2$ and $A_3$ are equal to zero. In Fig. 32 the steady state concentrations of ATP are plotted versus the normalized kinetic constant of the ATPase-reaction $\mathscr{H}_3 = k_3/k_1 S_1$ for various values of the normalized kinetic parameter of the leakage reaction $\mathscr{H}_4 = k_4/k_1 S_1$. The figure shows that this simple stoichiometric model possesses various properties found in the comprehensive model of glycolysis (see Section V.1). For small values of the leakage parameter $\mathscr{H}_4$ the steady state concentration of ATP is stabilized against variations of the ATP-consumption.

There exists a critical value $\mathscr{H}_3^c$ of the rate constant of the ATPase. For higher values of $\mathscr{H}_3 (\mathscr{H}_3 > \mathscr{H}_3^c)$ there exists only the steady state $A_3 = S_2 = 0$. Based on this model, Reich *et al.* (1976) analyzed various other reaction mechanisms which lead to ATP-production. They were particularly concerned with the question whether the models of various complexity show self-stabilization of the ATP-concentration. The models are depicted in Schemes 15a–c.

Scheme 15a represents a system where ATP is produced directly from another high energy compound $S_1$. The analysis of this model reveals that the ATP-concentration decreases strongly if the energy consumption is increased, i.e. no self-stabilization of the ATP-concentration is observed. In the reaction system represented in Scheme 15b, ATP is indirectly regenerated from a high energy intermediate $S_2$ which may also be degraded in a leakage reaction $v_4$. In this system the ATP-concentration remains relatively constant with variations of the energy consumption. This may be easily explained

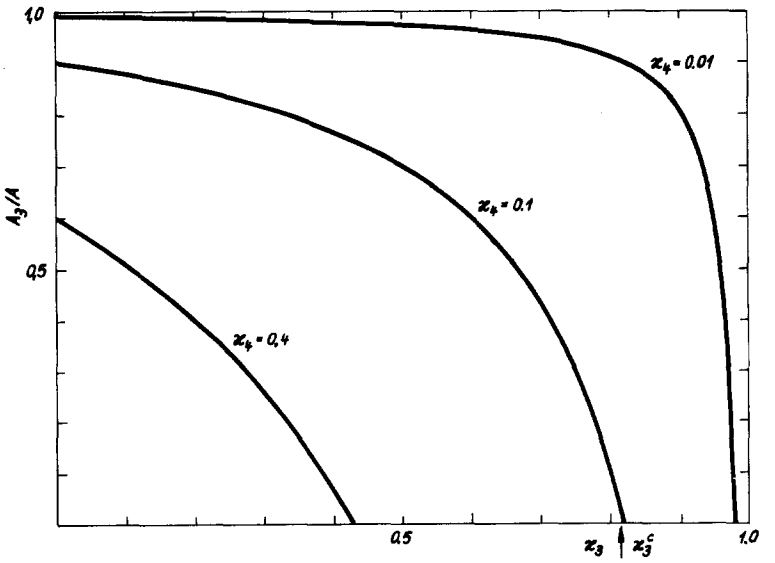
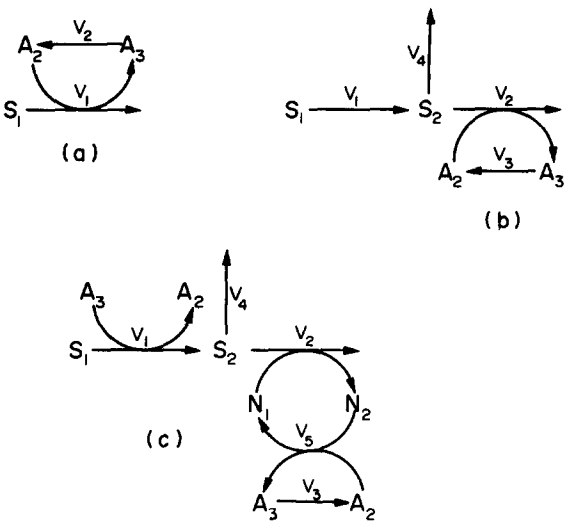



FIG. 32. ATP-concentration ($A_3$) as function of the normalized kinetic constants $\mathscr{H}_3 = k_3/k_1 S_1$ (ATPase) and $\mathscr{H}_4 = k_4/k_1 S_1$ (leakage) for the stoichiometric model of glycolysis depicted in Scheme 14. A denotes the total sum of the adenine nucleotides. $\mathscr{H}_3^c$ is the critical value of the kinetic constant of the ATPase. Parameter values: $\mathscr{H}_2 = k_2 A/k_1 S_1 = 5$, $\gamma_1 = 1$, $\gamma_2 = 2$.




Scheme 15.

by the fact that ADP which is produced by the ATPases drives the intermediate $S_2$ into the reaction $v_2$ in which ATP is produced, while the energy waste via the reaction $v_4$ is decreased. Scheme 15c represents a system with reflexive catalysis where ATP is indirectly regenerated via a reducing agent $N_2$. In Scheme 15c, $N_1$ stands for $NAD^+$ and $N_2$ for NADH. Reaction $v_5$ may be considered as a simplified representation of oxidative phosphorylation. This model yields a similar dependence of the ATP-concentration from the kinetic parameter of the ATPase reaction, i.e. self-stabilization of the ATP-concentration provided that the reaction $v_4$ is very fast.

Reich *et al.* (1976) pointed out the teleonomic behaviour of the model depicted in Scheme 14 in as much as at low ATP concentrations energy regeneration has precedence over the synthetic processes.

### 4. *Models to Describe Oscillations in Glycolysis*

Since periodic changes of glycolytic metabolites were first described in anaerobic yeast cells, numerous experimental and theoretical studies were carried out on oscillations in metabolic systems (for review of the experimental work see e.g. Hess *et al.*, 1969; Hess and Boiteux, 1971). In various types of cells and cell extracts the time dependence of metabolites was measured under differing conditions such as various pH-values and temperatures. Also the effects of various effectors of the enzymes of glycolysis on the occurrence and form of the oscillations were studied (e.g. Frenkel, 1968a).

Figure 33 shows a selection of periodic changes of metabolites in a cell free beef heart extract (Frenkel, 1968b). A characteristic feature of these oscillations is the reciprocal behaviour of the changes of fructose 6-phosphate and fructose 1,6-bisphosphate. This observation which was confirmed in many other studies led to the supposition that the phosphofructokinase plays a controlling role for the oscillatory behaviour. Two types of experimental results support this conclusion: Firstly, the observation that effectors of the phosphofructokinase influence the oscillations. They are suppressed, for instance, by the inhibitor citrate and stimulated by the activator inorganic phosphate (Frenkel, 1968a). Secondly, oscillations only occur by infusing of substrates which are metabolized via the phosphofructokinase. Accordingly, the vast majority of mathematical models of glycolytic oscillations are based on the assumption that the phosphofructokinase is the oscillator. The phosphofructokinase of most cells exhibits cooperative behaviour and is influenced by a multitude of allosteric effectors. Among them are the activators AMP, ADP, $P_i$ and fructose 1,6-bisphosphate and the inhibitors ATP, citrate and 2,3-bisphosphoglycerate.

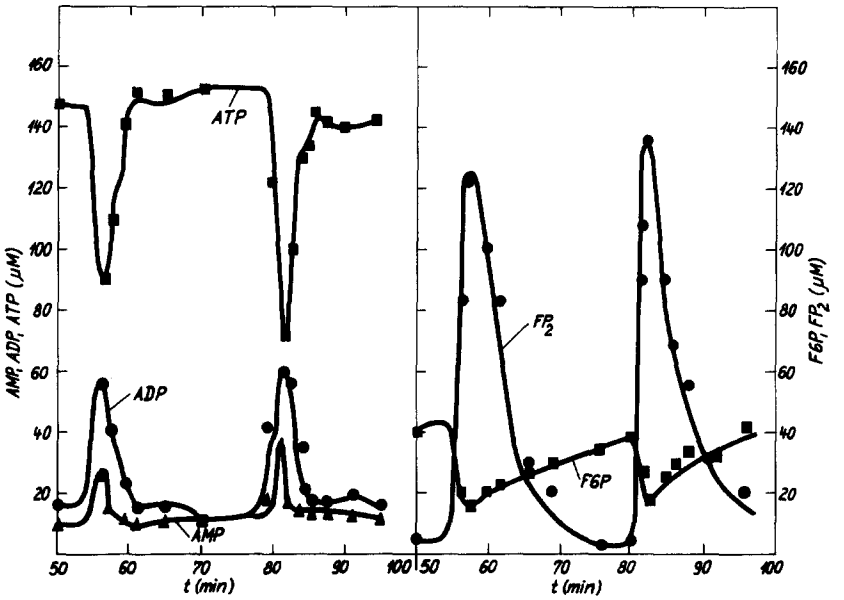
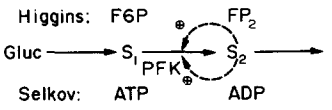



FIG. 33. Periodic changes of the concentrations of AMP, ADP, ATP and of the glycolytic intermediates F6P and $FP_2$ in cell free extracts of beef heart. The points are experimental data (Frenkel, 1968b) and the curves represent theoretical values obtained by Achs and Garfinkel (1968) by simulation studies.

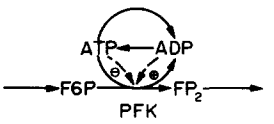
(a) *Models Based on Activation or Inhibition of Control Enzymes*

Higgins (1964) developed the first model which yielded a mechanistic interpretation of the oscillations in glycolysis. His model is based on a scheme of reactions which includes only the first enzymes of glycolysis (Scheme 16). It is assumed that there is a constant input into fructose 6-phosphate from glucose catalyzed by hexokinase and phosphoglycerate isomerase and that fructose 1,6-bisphosphate is removed by an irreversible reaction. This scheme includes an activating effect of fructose 1,6-bisphosphate on the phosphofructokinase. The model is a special variant of the back activation oscillator which has been considered in Section III.4.(a).(i). For certain combinations of parameters the system exhibits limit cycles around an unstable steady state. From the location of the limit cycle on the phase plane (Fig. 6), one can see that the model describes correctly the experimentally observed phase shift between fructose 6-phosphate and fructose 1,6-bisphosphate (see Fig. 33). A weakness of the model is the assumption of the constancy of the adenine nucleotides, which undergo significant changes in the experimental system (see Fig. 33).




Scheme 16.

Selkov developed several models for the description of glycolytic oscillations which presuppose the phosphofructokinase reaction as source of the oscillations. His first model (Selkov, 1968) resembles that of Higgins (1964) in assuming a linear chain of three irreversible reactions, in which the second one is activated by its product. In the reaction Scheme 16, $S_1$ stands for ATP and $S_2$ for ADP. In contrast to Higgins (1964), Selkov (1968) disregarded the activating effect fructose 1,6-bis-phosphate with the argument that under physiologic conditions the phosphofructokinase is saturated with this activator. Instead he takes into account the activating effect of ADP. The concentration of fructose 6-phosphate is assumed to be constant, which again does not correspond to the experimental data.




Scheme 17.

The basic model of Higgins–Selkov was supplemented further by Selkov (1972). Scheme 17 shows a model which takes into account additionally the phosphorylation of ADP to ATP and a nonglycolytic ATPase reaction. Oscillations are only observed if the influence of effectors on the phosphofructokinase, such as the activation by ADP or the inhibition by ATP are included.

Goldbeter and Lefever (1972) studied a model with similar essential features as the model of Selkov (1968). They introduced the special assumption that the cooperative properties of the phosphofructokinase are described by the model of Monod *et al.* (1965). By inclusion of diffusion processes spatially inhomogeneous structures could be generated.

In a later study it was shown (Boiteux *et al.*, 1975) that the entrainment of glycolytic oscillations by a periodic source of substrate is in excellent agreement with experimental results.

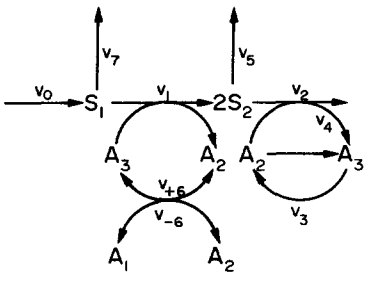
Dynnik and Selkov (1973) considered a model of the lower part of glycolysis. They showed that glycolytic oscillations may also arise theoretically by the activation of pyruvate kinase by fructose 1,6-bisphosphate or by the inhibition of the glycerinaldehyde-phosphate dehydrogenase by glyceraldehyde 3-phosphate. Both assumptions meet with difficulties. Under physiological conditions pyruvate kinase is saturated with fructose bisphosphate and a forward inhibition of the glycerinaldehydephosphate dehydrogenase by glyceraldehyde 3-phosphate has not been observed. They speculated on the possibility that the observed double periodicities in the oscillations of metabolites (Pye and Chance, 1966) may arise by interaction of an oscillator of the lower part of glycolysis with the phosphofructokinase-oscillator.

(b) *Oscillations in a Stoichiometric Model*

In this subsection there is considered in detail a model which is based on the glycolytic model proposed by Selkov (1975a,b). It demonstrates that oscillations arise in a System in which only stoichiometric interactions occur. In addition to the system shown in Scheme 14 the model represented in Scheme 18 takes into account (1) the irreversible input of glucose ($v_0$); (2) an alternative pathway of energy production ("respiration"), ($v_4$); (3) the adenylate kinase reaction ($v_{+6}, v_{-6}$); (4) an irreversible outflow of $S_1(v_7)$. The system is described by the following differential equations

$$\frac{dS_1}{dt} = v_0 - v_1 - v_7 \tag{5.17a}$$

$$\frac{dS_2}{dt} = 2v_1 - v_2 - v_5 \tag{5.17b}$$




Scheme 18.

$$\frac{dA_3}{dt} = -v_1 + v_2 - v_3 + v_4 - v_{+6} + v_{-6} \tag{5.17c}$$

$$\frac{dA_1}{dt} = -v_{+6} + v_{-6}. \tag{5.17d}$$

In addition there is the conservation equation for the adenine nucleotides

$$A_1 + A_2 + A_3 = A \tag{5.18}$$

For the metabolite dependent fluxes $v_1$ rate equations are used which are listed in Table 9. For the reactions 1 and 3, hyperbolic saturation functions are assumed with

TABLE 9. RATE LAWS ASSUMED FOR THE VARIOUS REACTION STEPS OF THE STOICHIOMETRIC MODEL OF GLYCOLYSIS (REACTION SCHEME 18)

| Reaction | Rate equation |
|---|---|
| 0 | $v_0 = \text{const}$ |
| 1 | $v_1 = \dfrac{k_1 A_3 S_1}{1 + A_3/K_1}$ |
| 2 | $v_2 = k_2 A_2 S_2$ |
| 3 | $v_3 = \dfrac{k_3 A_3}{1 + A_3/K_3}$ |
| 4 | $v_4 = k_4 A_2$ |
| 5 | $v_5 = k_5 S_2$ |
| 6 | $v_6 = k_{+6} \quad A_2^2 - k_{-6} A_1 A_3$ |
| 7 | $v_7 = k_7 S_1$ |

respect to the substrate $A_3$. For all other enzymes linear dependence on substrate concentration is assumed. It is presupposed that the reactions $v_2$, $v_{+6}$ and $v_{-6}$ are very rapid in comparison with the others. In this case the steady state approximation may be applied to the metabolites $S_2$ and $A_1$. From

$$2v_1 - v_2 - v_5 = 0 \tag{5.19}$$

one obtains with the kinetic equations from Table 9

$$S_2 = \frac{2k_1 S_1 A_3}{\left(1 + \dfrac{A_3}{K_1}\right)(k_2 A_2 + k_5)}. \tag{5.20}$$

The steady state approximation for $A_1$,

$$v_6 - v_{-6} = 0, \tag{5.21}$$

leads to the equilibrium condition for the adenylate kinase, which has been given in Eqn. (5.9). Because of the validity of the conservation equation for the adenine nucleotides (Eqn. (5.18)) both $A_1$ and $A_2$ may be expressed as unique functions of $A_3$ (see Eqns. (5.10a,b)). Under such assumptions the system of differential equations reduces itself to two equations

$$\frac{dS_1}{dt} = v_0 - k_7 S_1 - \frac{k_1 S_1 A_3}{\left(1 + \dfrac{A_3}{K_1}\right)} = f_1(S_1, A_3) \tag{5.22a}$$

and

$$\left(2 - \frac{dA_2(A_3)}{dA_3}\right)\frac{dA_3}{dt} = \frac{k_1 S_1 A_3 (k_2 A_2(A_3) - k_5)}{\left(1 + \frac{A_3}{K_1}\right)(k_2 A_2(A_3) + k_5)}$$

$$- \frac{k_3 A_3}{1 + \frac{A_3}{K_3}} + k_4 A_2(A_3) = f_2(S_1, A_3). \tag{5.22b}$$

The dynamic properties of the system may be investigated in the phase plane. One obtains for the quasi-steady state lines from Eqns. (5.22a,b)

$$S_1^{\mathrm{I}} = \frac{v_0}{k_7 + \frac{k_1 A_3}{1 + \frac{A_3}{K_1}}} \tag{5.23a}$$

$$S_1^{\mathrm{II}} = \frac{\left(\frac{k_3 A_3}{1 + \frac{A_3}{K_1}} - k_4 A_2\right)(k_2 A_2 + k_5)\left(1 + \frac{A_3}{K_1}\right)}{(k_2 A_2 - k_5) k_1 A_3}. \tag{5.23b}$$

Figure 34a,b shows the quasi-steady state lines for different values of the kinetic parameters. Whereas the line $S_1^{\mathrm{I}}(A_3)$ shows a simple monotonic course, the line $S_1^{\mathrm{II}}(A_3)$ is rather complicated. It consists of two branches ($a_1$, $a_2$ and $b_1$, $b_2$, Fig. 34b). Along $a_1$ and $b_1$ both the numerator and denominator in Eqn. (5.23b) are negative and along $a_2$ and $b_2$, positive. Since along the branches $a_2$ and $b_2$ energy consumption is greater than energy production by respiration ($v_3 > v_4$), these branches describe possible real conditions with glycolysis as an energy producing system. The condition characterized by the branches $a_1$ and $b_1$ corresponds to an excess of energy production by respiration over degradation by ATPases ($v_3 < v_4$), and glycolysis would have to be an energy consuming process. The ATP-balance is achieved in this case by a predominant flux of glycolysis through the sidepath ($v_5 > v_2$). In the reaction $v_2$ consequently less ATP is produced than is used in the reaction $v_1$.

The steady state of the system lies on the intersection of the lines $S_1^{\mathrm{I}}$ and $S_1^{\mathrm{II}}$. Because of the complicated course of line $S_1^{\mathrm{II}}$ there may exist several steady states simultaneously. It is of particular interest that the system may exhibit relaxation oscillations if $A_3$ is a rapid variable and if the system possesses unstable steady states (see Section III.5.(c).(i)).

For the stability analysis one utilizes the methods described in Sections III.2 and III.4. The easiest way to find the bifurcation points in the present case is to vary the parameters $v_0$ and $k_7$, since they influence only the position of the quasi-steady state line $S_1^{\mathrm{I}}$ but not that of $S_1^{\mathrm{II}}$. In Fig. 35 is shown the $(k_7, v_0)$-plane which is divided by the bifurcation lines $\mathrm{tr}(v_0, k_7) = 0$ and $\Delta(v_0, k_7) = 0$ into three domains. In the domain A the system possesses only a single stable steady state. With the passage into domain B, two additional steady states arise, one a stable steady state, the other an unstable saddle-point. When crossing from the domain A to C, an unstable state is produced surrounded by a limit cycle (Hopf bifurcation).

In Fig. 36 are shown for parameter values which had been taken from the domain C two different trajectories, which merge with the limit cycle from inside or outside.

The stoichiometric model is of importance for the theory of metabolic regulation, since it shows that periodic changes of metabolites may occur in models which do not include activation or inhibition of enzymes by metabolites. To achieve periodicity non-glycolytic ATP-producing and -consuming reactions have to be included. A detailed analysis shows that the model system exhibits relaxation oscillations only if (1) the

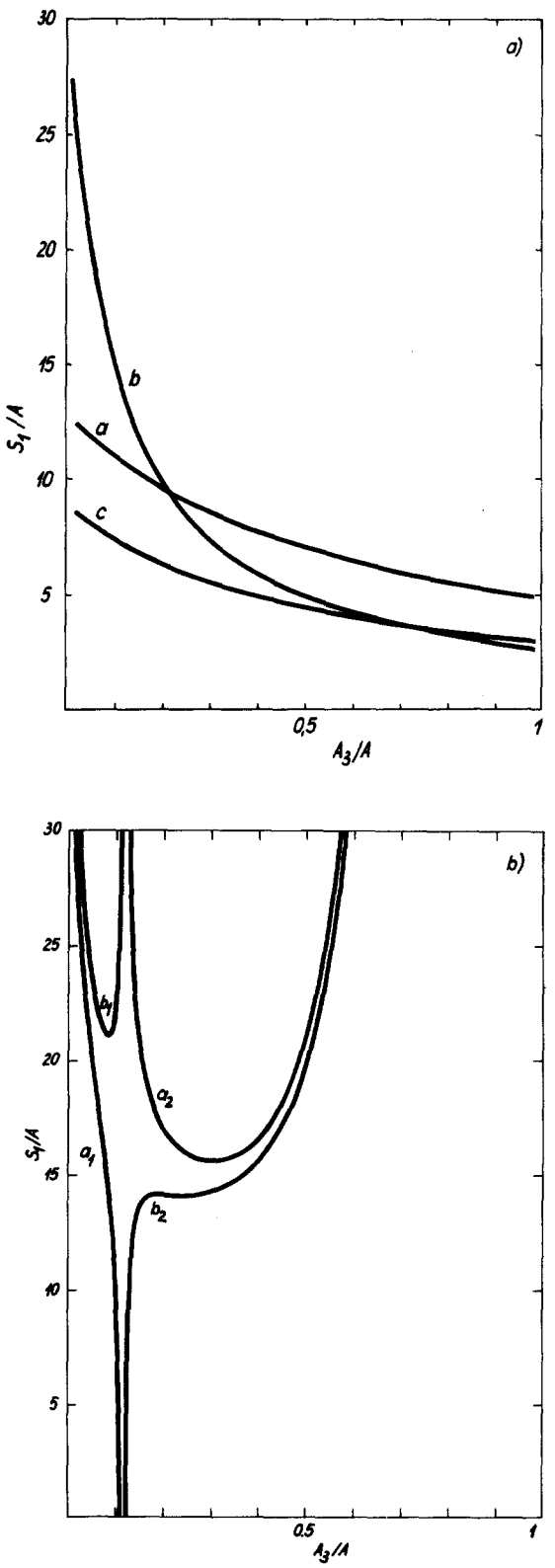



FIG. 34. Quasi-steady state lines of a stoichiometric model of glycolysis (reaction Scheme 18). (a) steady state line $S_1^{I}(A_3)$ (see eqn. (5.23a)) dimensionless parameter values: $K_1/A = 10^2$; curve a: $v_0/k_1A^2 = 7.8$, $k_7/k_1A = 0.6$; curve b: $v_0/k_1A^2 = 3.0$, $k_7/k_1A = 0.1$; curve c: $v_0/k_1A^2 = 4.5$, $k_7/k_1A = 0.5$. (b) The steady state line $S_1^{II}(A_3)$ consists of two branches ($a_1$, $a_2$ or $b_1$, $b_2$, see eqn. (5.23b)). Dimensionless parameter values: $K_1/A = 10^2$, $K_3/A = 0.5$, $k_3/k_1A = 8.5$, $k_4/k_1A = 0.2$, $k_5/k_2A = 3.9$ (for the branches $a_1$, $a_2$), $k_5/k_2A = 4.1$ (for the branches $b_1$, $b_2$).

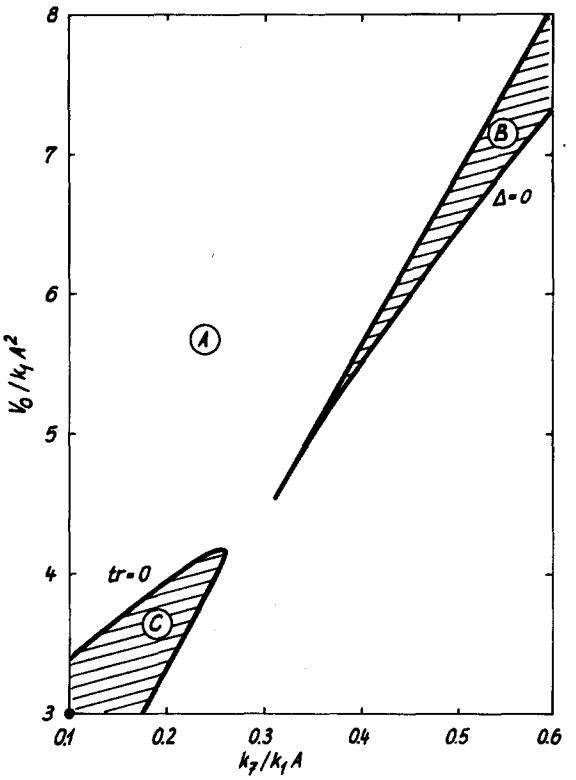



FIG. 35. Parameter regions corresponding to steady states with different stability characteristics and different number of steady states for the stoichiometric model of glycolysis (reaction Scheme 18). Region A: unique stable steady state. Region B: three steady states for each parameter set. Region C: unique unstable steady state. The following dimensionless parameter values were used: $K_1/A = 0.5$, $K_3/A = 10^2$, $k_3/k_1A = 8.5$, $k_4/k_1A = 0.1$, $k_5/k_2A = 2.0$.

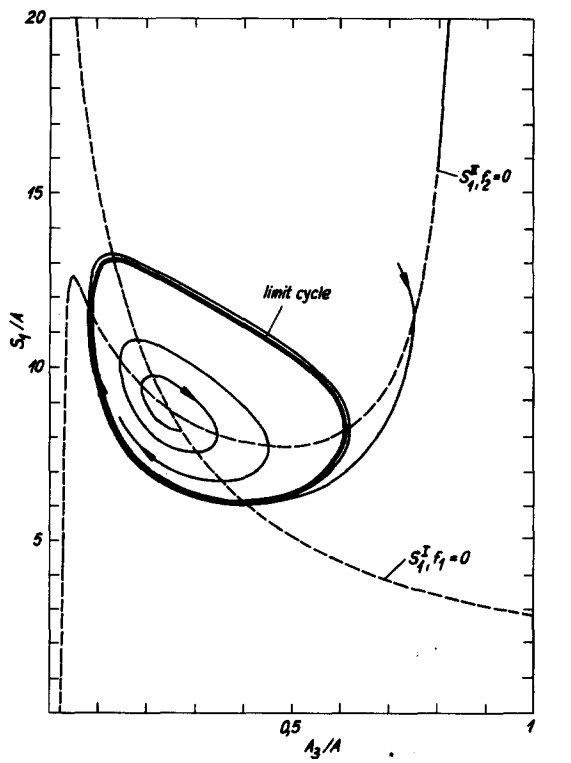



FIG. 36. Trajectories of the stoichiometric model of glycolysis (reaction Scheme 18) which approach a limit cycle. Parameter values: $v_0/k_1A^2 = 3.0$, $k_7/k_1A = 0.1$. The other parameter values are as indicated in the legend to Fig. 35.

velocity of the enzyme $E_1$ depends on the concentration of $S_1$ and (2) if the variable $A_3$ is fast in comparison to $S_1$. Generally, in real systems both requirements are not fulfilled simultaneously. In cells with high glucose concentration (e.g. erythrocytes) ATP is a faster variable than glucose but the hexokinase is saturated by glucose, i.e. the first requirement is not fulfilled. In other cells with low concentrations of glucose (e.g. muscle) the velocity of the hexokinase depends on the glucose concentration but ATP is the slow variable. It seems, therefore, that the explanation of oscillations in real systems requires the inclusion of non-stoichiometric couplings, such as the activation of phosphofructokinase by AMP or ADP.

All models considered so far for biological oscillations may be considered as skeleton-models, which try to account for various essential features of glycolysis. Their advantages are the small number of differential equations required and the possibility to prove the existence of limit cycles in a rigorous manner. The large number of models proposed indicates that they are not unique and that there is an element of arbitrariness in their construction.

An opposite approach was chosen by Achs and Garfinkel (1968). For the simulation of the experimental data of Frenkel (1968b) they utilized a system of fifty-seven differential equations and included in a detailed manner the cooperative properties of the phosphofructokinase. A satisfactory simulation could only be achieved if a complicated time dependency of the kinetic parameters of the ATPases was assumed which was imposed from outside on the system, or an activation of the ATPases by 1,3-bisphosphoglycerate, for which there is no biochemical evidence. According to the great number of kinetic parameters of the enzymes and corresponding to their strategy of fitting the solutions to the experimental data, the results were not unique. Because of the high dimensions of the model it could not be shown whether the oscillatory solutions represent stable limit cycle or transitory periodicities.

Richter *et al.* (1975) also used a detailed model to investigate the influence of perturbations of the NADH/$NAD^+$-system on the oscillatory behaviour of the glycolytic intermediates. For all enzymes, including the very fast ones, kinetic equations were used. The oscillations were generated by the activating effect of fructose 6-phosphate and the inhibiting effect of ATP on the phosphofructokinase. The model could simulate the suppression of oscillations by a sudden decrease of the NADH-level which was induced by addition of acetaldehyde (Pye, 1973).

### (c) *The Significance of Oscillations in Metabolism*

The observation and theoretical investigation of oscillations in metabolism has had an extraordinarily stimulating effect on various fields of biology. The biochemical experimenters have presented a number of examples which show under special conditions self-sustained oscillations ranging the whole gamut from simple enzymes systems, cell and tissue extracts to intact cells. Oscillatory phenomena have been known of course for a long time in various complex biological systems as well as in simple chemical ones. Their analysis, however, had not yielded a deeper understanding of the molecular basis of biological periodicities. This has been provided by the biochemical studies.

Theorists have furnished a wide choice of reaction schemes for which the existence of limit cycles could be proved. At present there is no longer any lack of theoretical skeleton models. In principle the occurrence of limit cycles is no longer surprising since metabolic networks are represented by non-linear differential equations. The problem now is rather, which of the variety of models are applicable to a given biological system and under which biologically realistic conditions. Two biological questions may be asked:

(1) Are metabolic networks designed in such a way as to suppress limit cycles or rather to generate them? Or is there a middle ground, i.e. that limit cycles represent a special adaptation?

(2) Have oscillatory mechanisms developed in such a manner as proposed by Selkov (1975a) that the stoichiometric regulation constituted a more primitive and more ancient stage than allosteric regulation?

With respect to the first question, it has been noted before that biological systems and metabolism in particular are characterized both by features of great stability and of variability which sometimes assumes the character of periodicity. The principles of homeostasis which have developed during evolution tend to stabilize and smooth all parameters of living systems, be they nutrients, pH and other ions or temperature. Therefore, the main direction of evolution of design appears to be towards the stabilization of metabolic networks. On the other hand, there is no denying the fact that periodicities pervade all of living nature, and become prominent in particularly developed specialized systems, such as nerve cells. Obviously we are faced with a dialectic unity of two contradictory aspects of the organization of living systems. Thus one may give a general affirmative answer to the question posed. Apparently metabolic systems may be designed to exhibit both features of stability and of periodic behaviour. Whether oscillation have a definite importance on the level of intermediary metabolism itself may well be questioned. Their usually submerged oscillatory faculties rather may serve as elements to be used in more complex functions of biological systems, such as cell division, differentiation, circadian rhythms and the like (Goodwin, 1973; Tyson and Kauffman, 1975). As far as the second question is concerned a general answer may not emerge. There are reasons to doubt that in the course of biological evolution allosteric regulation has appeared much later than the stoichiometric one. One does not observe in nature a progressive development of allosteric properties of enzymes. Regardless of their position in phylogeny enzymes of bacteria, or low eucaryotes give no indication of lesser allosteric control than those of mammals or man.

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