THE CO-ORDINATION OF CHEMICAL PROCESSES IN THE LIVING CELL

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INTRODUCTION

During my last visit to Madison I saw on Van Potter's desk a calendar with the question "Are you helping with the solution, or are you part of the problem?" This dilemma is a suitable description for the phenomenon of co-ordination. You will probably become convinced of this after what I have to say, but it is also applicable to the action on the organism of drugs, the preparation of which is your job.

In numerous meetings recently, the problems of co-ordination and regulation of metabolism have been discussed[1–6]. What is the best use for our next hour? I finally decided to draw up a catalogue of fundamental principles. No point will be dealt with thoroughly, but nevertheless each single point will be illustrated with a number of interesting experimental details taken from literature on the subject and partly from experiments carried out by my colleagues Hohorst, Kadenbach and Schimassek.

Let us first discuss a plan of metabolism, that is the plan of a system of co-ordinated chemical reactions.

CO-ORDINATION OF REACTIONS

The simplest unit of the cellular chemical organization is a reaction between two partners, the *two-partner reaction.*

$$A \underset{\text{Enzyme}_{AB}}{\rightleftharpoons} B$$

The metabolites A and B are necessarily isomers and the reaction is catalysed by an enzyme. We may take as an example triosephosphate isomerase (*Figure 1*), the enzyme of the Embden–Meyerhof chain which can be crystallized from muscle[7]. About ten thousand molecules of ketotriose and aldotriose per second are brought to equilibrium by a molecule of this enzymatic protein.

From single reactions such as the one described, there follows a sequence of reactions when our enzymatic reaction shares one of its partners with another reaction. There is then obtained, in the simplest cases of a system of co-ordinated chemical reactions, a chain of two-partner reactions.

$$O \underset{\text{Enzyme}_{OA}}{\rightleftharpoons} A \underset{\text{Enzyme}_{AB}}{\rightleftharpoons} B \underset{\text{Enzyme}_{BC}}{\rightleftharpoons} C \ldots$$

Biochemists call this linking of chemical reactions by means of common reaction partners "coupling". In practice, the setting up of this relationship

has the same meaning as the passing from a state of building stones to that of organized building. Depending on the point of view, coupling is called chemical or energetic; the basic fact, that a metabolite takes part in more than one reaction, remains the same.

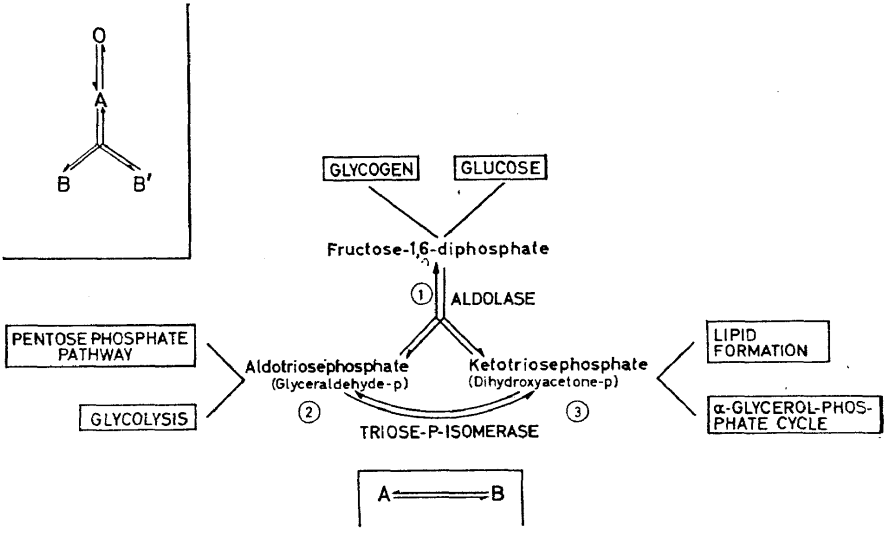
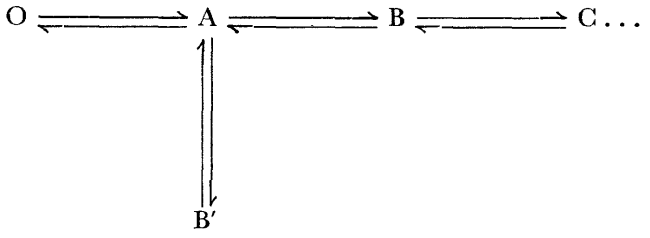


Figure 1. Co-ordination of a three-partner reaction (aldolase) and a two-partner reaction (triosephosphate isomerase) at the key position of glycolysis; for connection to other systems see *Figure 3*, point 1, and *Figure 5*, points 2 and 3

There is a branching in the chain of reactions if one of the intermediary substances enters as a partner in more than two reactions.



With this branching the system gains new degrees of freedom. This can be seen by considering the flow velocity in the steady state: in the unbranched metabolic chain it is sufficient to determine the flow velocity of only one of the two-partner reactions to obtain the others.

$$V_{OA} = V_{AB} = V_{BC}$$

In the simple branched chain there are already three different flow velocities.

$$V_{OA} = V_{AB} + V_{AB'}$$

We have seen an increasing number of reactions co-ordinated by the very same metabolite; the number of steady state flow rates also increases. As

an example with a practical significance we can take the measurements of Ashmore *et al.*[8] on liver slices from normal and from diabetic rats (*Figure 2*): in the liver the intermediary metabolite, glucose-6-phosphate, is a partner in five reactions (a-e in *Figure 3*). The velocity of one of these reactions, the synthesis of this metabolite by phosphorylation of glucose (a), is represented

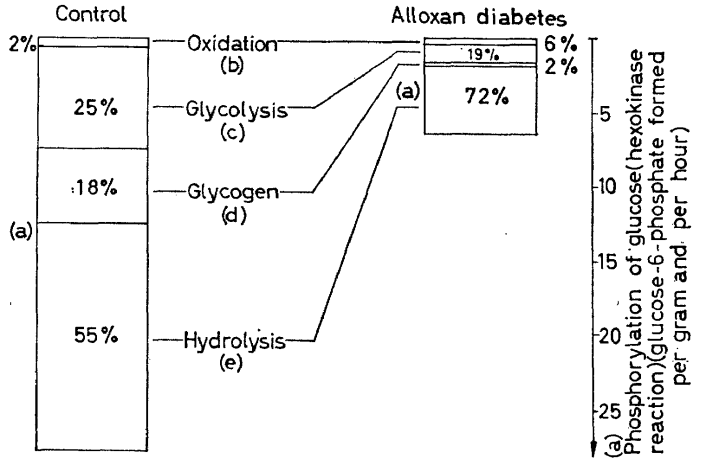


Figure 2. Turnover of glucose-6-phosphate in liver slices in high K^+-medium from normal and alloxan diabetic rats; data taken from Ashmore *et al.*[8]

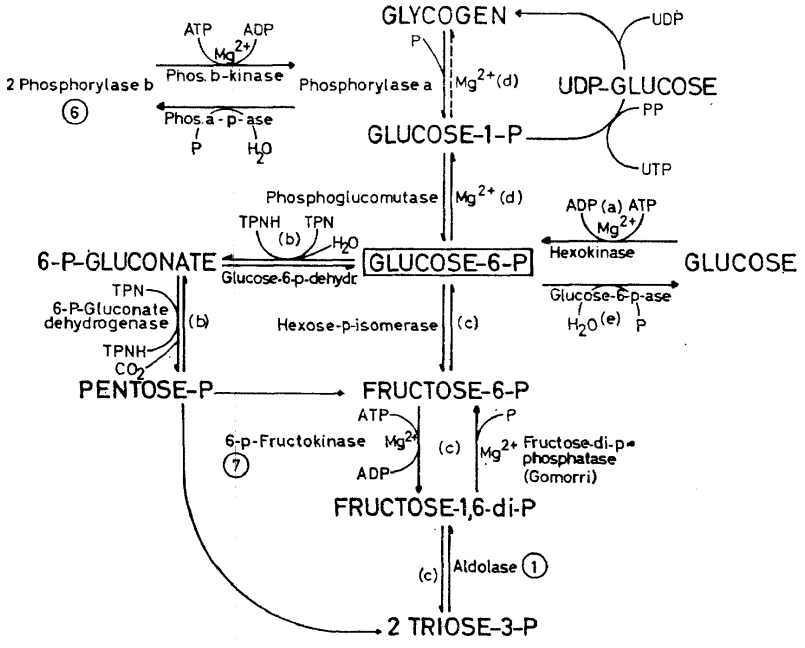
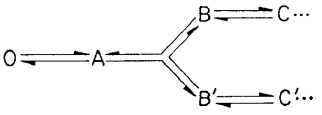


Figure 3. Metabolic pathways of glucose-6-phosphate

in *Figure 2* by the size of the blocks. The blocks are subdivided proportionately to the partnership of the other four reactions (b-e). The metabolites linking several metabolic reactions are also sometimes called "key metabolites". This example shows how an understanding of metabolic structure serves as a rational basis for the understanding of the metabolic behaviour of a liver slice in a case of alloxane diabetes.

With a *three-partner reaction* another type of branching can be obtained.



These reactions introduce "dichotomic" ramifications; according to the strict rules of stoicheiometry the fission products B and B′ are formed and recombine at the same velocity.

A typical example for such a three-partner reaction is aldolic splitting of fructose diphosphate catalysed by the enzyme aldolase in *Figure 1*. The breakdown products, phosphorylated keto- and aldotriose, are formed in equal molecular proportions. Each of the two trioses possesses special metabolic relationships: aldotriose with the pentose phosphate network and with the phosphorylative oxido-reduction of the Embden–Meyerhof pathway, and ketotriose with glycerophosphate and the reactions connected with it. On bifurcation the metabolic flow should therefore split into two equal parts, unless ketotriose and aldotriose are balanced by the active and widespread triose isomerase. Thus, from the type of reaction and the organization characteristics the widespread partnership of aldolase and triosephosphate isomerase in all living cells can be understood.

A biological understanding of intermediary metabolism mechanisms began when Warburg and Meyerhof[9, 10] discovered the four-partner reactions of hydrogen and phosphate transfer (*Figure 4*). In the four-partner (symmetric) reaction two streams meet: the reaction connects, therefore, two metabolic pathways. Since the number of the initial and final products of the intermediate metabolism is relatively limited, whereas the number of the four-partner reactions is relatively high (about half of all the reactions of metabolism) it follows that one of the above pathways belongs to a cycle. There belongs to the same cycle also a corresponding four-partner reaction which moves in an opposite direction: it may be part of the same metabolic chain or part of another different chain.

In order to give an example, *Figure 5* summarizes the hydrogen transfer reactions which are localized in the liver extramitochondrial compartment. In these reactions (3-5), on the substrate side, a keto group is converted into a secondary alcohol group, or vice-versa (*cf. Figure 4*), and in these reactions the hydrogen acceptor is diphosphopyridine nucleotide and the donor is its reduced form. The details of reaction (2) are somewhat more complicated (five-partner reaction) and cannot be discussed here.

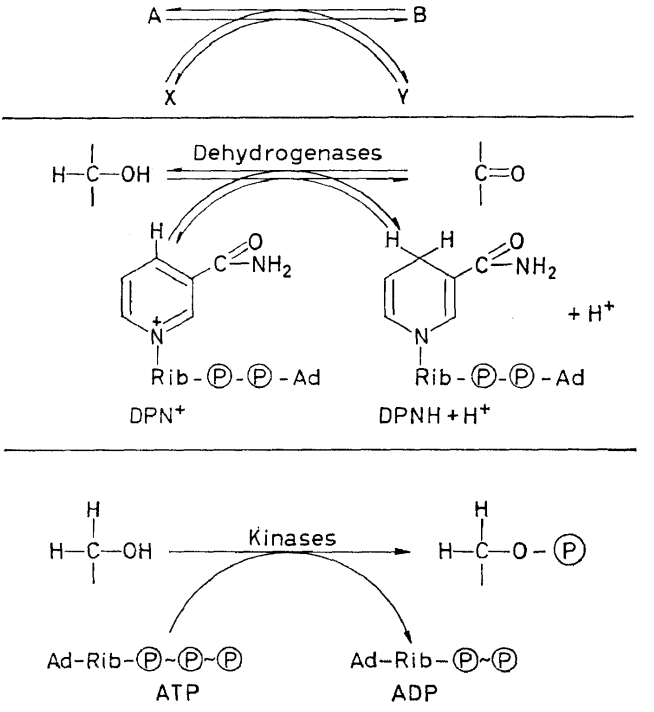


Figure 4. Four-partner reactions transferring hydrogen (dehydrogenases) and phosphate (kinases)

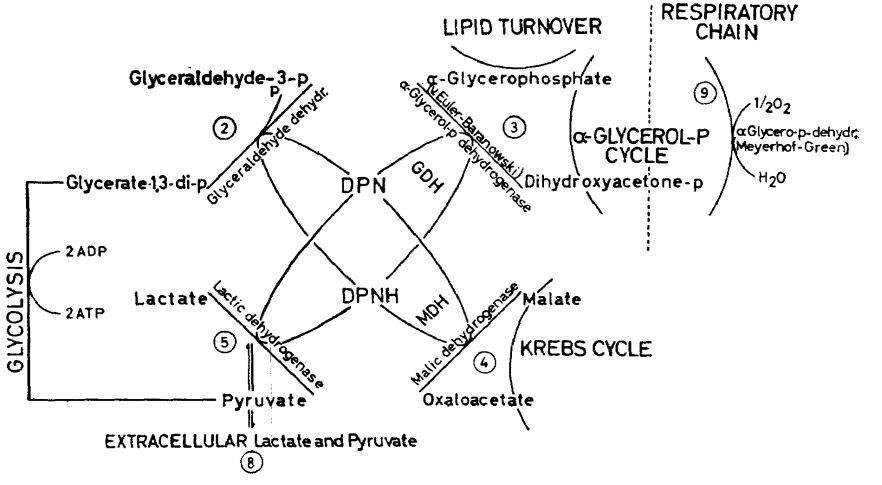


Figure 5. Redox relations of the extramitochondrial DPN-system; for connection with other systems see *Figure 3*, points 2 and 3; it connects also with the mitochondrial respiratory chain at point 9 and with the redox-state of the extracellular spaces at point 8

The dehydrogenases in *Figure 5* have a high activity and belong, as already stated, to the same intracellular compartment. Therefore, as far as the nucleotide is concerned, they draw from, and contribute to, the same pool of hydrogen. But, in text-books, these reactions are found in different chapters, the first and second are in a description of the glycolysis chain, the third in the citric acid cycle, the fourth in the lipid synthesis or also in a discussion on reactions proposed for a glycerophosphate cycle (see below). And yet from the point of view of the co-ordination of metabolic systems these reactions must be considered linked. They couple the metabolic systems. I shall come back later to this example. It is enough just now to say that analogous systems of "transport metabolites" acting in "four-partner" reactions set up an almost incalculable number of correlationships between the different cell systems. It is this type of coupling, especially when it co-ordinates parts of the same chain, that is meant when the idea of "feed-back" is used, quite expressive but not too successful from a theoretical point of view[11].

The common correlating partners are mainly the free nucleotides, and nowadays most of their transport-functions in metabolism are known[12] (see also refs. 17 and 18). But they can also be ordinary metabolites: the pentose phosphate network is an example of a network of four-partner reactions without any participation from free nucleotides[13, 14].

I shall limit myself to naming only the *five-* or *six-partner reactions*. To what has been explained above there should be added the coupling between systems. As an example, the Lynen–Johnson hypothesis of the Pasteur effect may be cited[15, 16]. Apart from exceptions these components are to be found in the so-called multi-enzyme systems, which cannot be discussed in this lecture (see refs. 17 and 18).

CO-ORDINATION OF PROCESSES

In the previous discussion of the plan of organization the type of metabolism was emphasized. If now we turn to the metabolic capacity, a quantitative treatment must be used. A characteristic of this different approach to the problem is the fact that an exact definition of the functional state of cells and tissue is a necessary basis for research.

An example of these slightly abstract statements is given in *Figure 6*. This shows the flow rate of lactate production of skeletal muscle (rat abdominal muscle) during the transition from the resting state to a tetanic contraction. It can be seen that the velocity of lactate formation increases a thousandfold.

Qualitatively, the process, both in the resting and in the active state, can be described by the well-known Embden-Meyerhof pathway. Its *quantitative* aspects can be taken as an example of the general phenomenon of the co-ordination between physiological and metabolic function.

Firstly, as for metabolic coupling, this co-ordination too is brought about through partners common to two processes (*Figure 7*). Thanks to the idea of coupling between supply and utilization in a "free market" of chemical energy, biochemistry has made extraordinary progress, especially after Lipmann's adaptable formulation of the concept of "energy-rich phosphate"[20]. According to this the myofibrillar contractile system is assumed to split

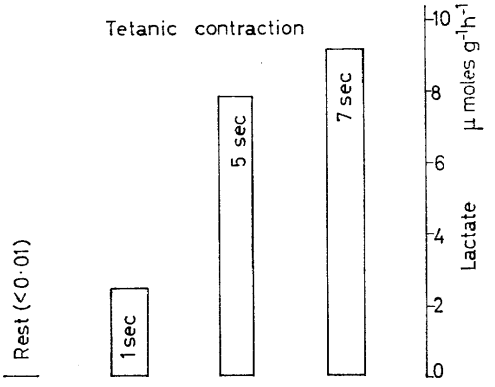


Figure 6. Flow rates of lactate production in the rat abdominal muscle in rest and during the first seconds of tetanic contraction[19]

energy-rich phosphate, and the glycolysis system to perform the opposite reaction. Since the pool of phosphate and phosphate acceptors in the cell is limited, coupling becomes quite strict[21]. When oxygen is supplied in sufficient amount, there are analogous relationships between work and the consumption of oxygen in muscle[22] (and see discussion in ref, 2).

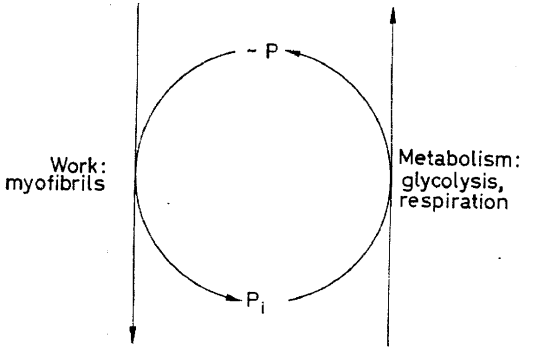


Figure 7. Co-ordination by the phosphate cycle of energy-metabolism and work in muscle

In addition to the phenomenon of physio-metabolic interdependence our experiment (*Figure 6*) shows a second interesting feature of the system under investigation: large variations in the flow velocity are going on in a short time. The question arises as to how this abrupt modification can be reconciled with the complexity of the glycolysis system. Certainly the glycolytic system cannot be switched on and off like an electric circuit, because operation of the metabolic network depends on the steady state pattern of some twenty metabolites, which, due[23] to manifold linkages, cannot be built up instantaneously. To cut a long story short we can take a look at the object of our investigation. *Figure 8* shows us metabolite levels of the glycolytic

chain in the resting state and during tetanic contraction. These figures show a great resemblance between the two patterns: in all the intermediate stages of the glycolytic chain the concentrations in the resting state are not substantially different from those in a state of action, with a flow rate a thousand times greater. Thus we see that the metabolite pattern of action is built up

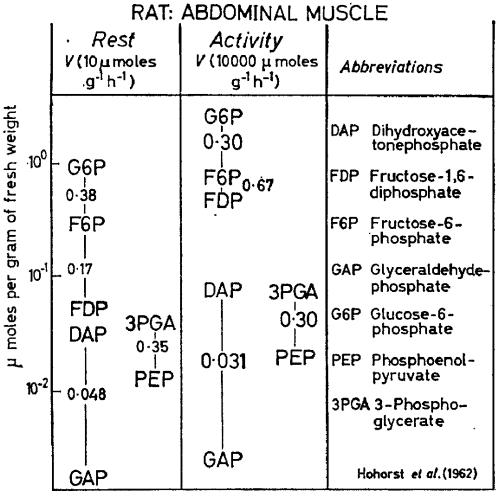


Figure 8. Metabolite levels of the glycolytic chain on the resting state and during tetanic contraction; logarithmic scale, ratios of levels between metabolites[19]

almost perfectly even during the resting state. In the terminology of our analogy for economy we might say that the producers prepare themselves in a depression for a new campaign in all the intermediate stages of production. It appears that this new phenomenon of co-ordination is found in all tissue biologically equipped for rapid changes of essential qualities. This was described for the first time after research on metabolism in the flight muscle of insects[23].

THE LAW OF MASS ACTION

The deviations of the cellular metabolite levels observed in our experiment are several times smaller than the variations of the flow velocity, and for some metabolites they really fall within the limits of error of analytic determinations. What then are the factors that act in this way in the stabilization of the pattern of intermediate metabolites? To answer this question a deeper insight into the phenomenon of metabolic control is needed.

From an examination of our patterns, the importance of the law of mass action is at once apparent (*Figure 9*). It can be seen without complications in the case of two-partner reactions[19] (catalysed by mutases, by isomerases and by enolase). The proportions of the metabolite contents in relaxed muscle are remarkably near the equilibrium constants according to the law of mass action ($\bar{Q}$ in *Figure 9*). In the transition of the tissue to action (*Figure 8*) the proportions do not change substantially.

The high activity of the cellular enzymes concerned is responsible for this phenomenon, with respect to the flow velocity of metabolism. In the state of activity the proportions of these reactions are only slightly shifted with respect to static equilibrium. Alberty has shown how this shift can be connected to the kinetic constants[23, 24]

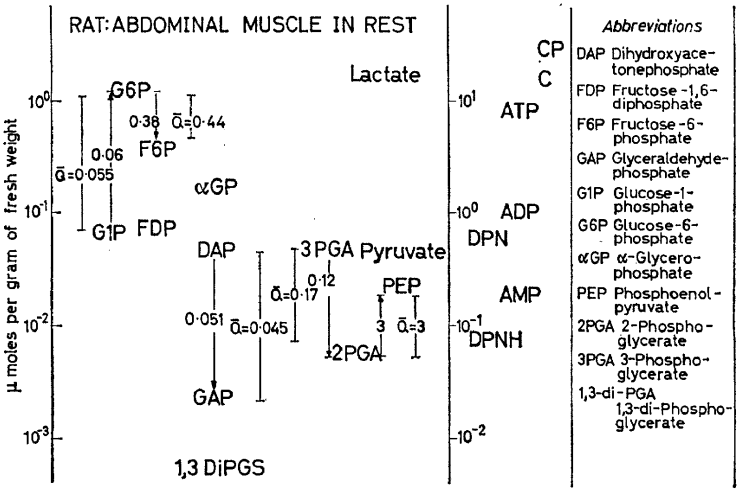


Figure 9. Metabolite levels of rat abdominal muscle in rest[19] (see also unpublished results); logarithmic scale, metabolites treated as in *Figure 8*, ratios of mass action equilibrium are also given (Q)

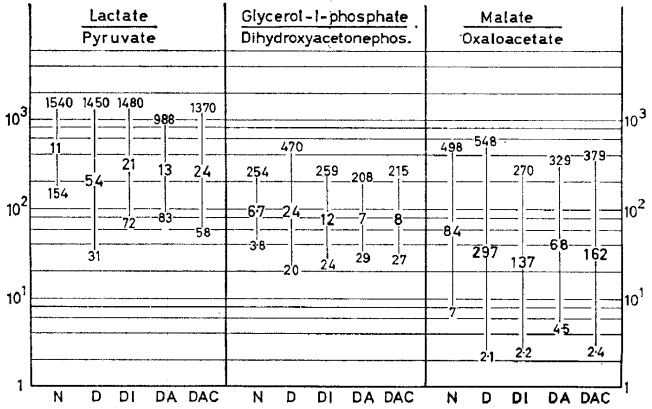


Figure 10. Metabolite levels (DPN-system) of rat liver in the normal state and under different stimuli on the endocrine regulation system[25]
N = normal, DI = diabetes plus insulin, D = alloxan diabetes, DA = diabetes plus adrenectomy, DAC = DA plus cortisol
In each state the proportions of the ratios measured approximate to the proportion of mass action equilibrium constants. $K_{lac.} : K_{glyc.} : K_{mal.} = 10 : 6{\cdot}2 : 120$ (see text)

A remarkable approximation to the equilibrium of mass action is to be found not only in the two-partner reactions. It has been recently shown with experiments on liver (*Figure 10*) that the proportions between pyruvate and lactate, malate and oxaloacetate, glycerophosphate and dihydroxyacetonephosphate are fairly constant[25–27]. The coupling between these oxido–reduction reactions by means of the pyridinenucleotides system has already been discussed (3–5 in *Figure 5*). Research on the metabolite contents show that the proportions of these oxido–reduction couples are correlated to the equilibrium constants of their transhydrogenation reactions. The figure shows this in a normal state and under different stimuli in the endocrine regulation system.

It is remarkable that for two fundamental transamination reactions these changes also occur[28]. Here too it seems that the law of mass action is decisive in fixing the concentrations of metabolites, the glucogenic amino-acids (*Figure 11*).

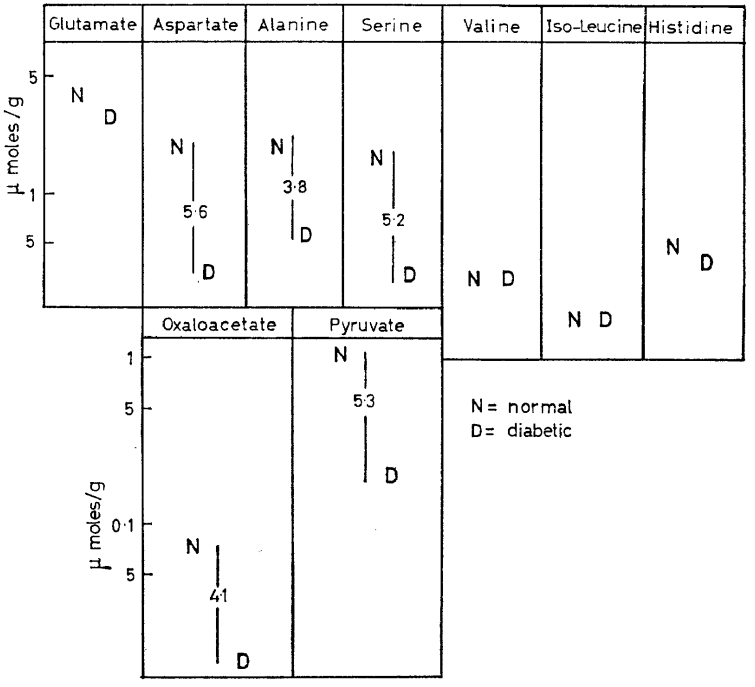


Figure 11. Levels of glucogenic amino-acids, oxaloacetate and pyruvate in normal and diabetic rat liver[28]

HIGH ENZYME ACTIVITIES

The condition of non-equilibrium is an indispensable basis for variability and control of living matter. Therefore, not all reactions of a metabolic system can take place in a state close to static equilibrium. Nevertheless, the proportion of these reactions in cell metabolism is high (about 2/3). It

includes all those reactions whose corresponding enzymatic activity is not controlled and (when tested under standard conditions[29, 30]) exceeds substantially the value of the metabolic flow velocity.

The diminution or increase in the activity of these enzymes, obtained with drugs or hormones, has no influence on the metabolic events in the cell, at least as far as the order of magnitude is concerned. The attempt which is often made to relate small variations of the activity of these enzymes to the mechanism of a drug action is basically wrong. Davenport[31] clarified this point recently with respect to the pharmacology of carbonic anhydrase (*Figure 12*).

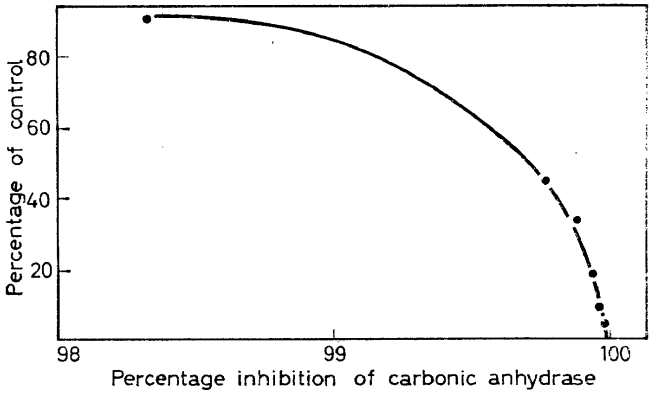


Figure 12. Ordinates: catalysed rate of uptake of carbon dioxide by dog red blood cells (percentage of control)
Abscissae: percentage inhibition of carbonic anhydrase
Enzyme inhibition was caused by addition of thiophene-2-sulphonamide to the reaction mixture, and the percentage inhibition was calculated from data studies of enzyme-inhibitor reaction mixtures; catalysed rate of uptake was estimated by substracting the rate observed when increase in inhibitor concentration caused no further diminution in uptake from the observed rate of uptake (data from Davenport, 1945)

CONTROLLED NON-EQUILIBRIUM

A characteristic of the group of metabolic reactions just named is that the proportions of the metabolites increase, when the flow velocity increases, away from the state of equilibrium (group A in *Figure 13*). These reactions can be contrasted with the few but decisive reactions that are in a state of controlled non-equilibrium (group B in *Figure 13*). Their activation produces an increase in the flow rate and the reaction system comes nearer to equilibrium.

The network of energetic metabolism has been compared to a network of regulated waterways, in which the individual sections on a constant level are connected by floodgates. Following up this analogy, we can say that, so far, with the reactions of group A, only stretches of canals have been described, and that now we must turn to the floodgates. In principle this is the case[32, 33]; but models are useful only when not too much is brought to bear on them. Metabolism dynamics is fundamentally different from waterways dynamics: the former, as has already been stated at the beginning, is connected to the laws of stoicheiometry.

In the intermediate metabolism control zones it is first and foremost a question of the regulation of enzyme activity. What is known of the mechanism acting in this way is not yet complete. It is fairly clear, however, that in many essential control points of energetic metabolism the state of non-equilibrium existing in the cell between inorganic phosphate and its organic compounds is involved. In almost all these reactions magnesium and/or calcium cations have a part to play.

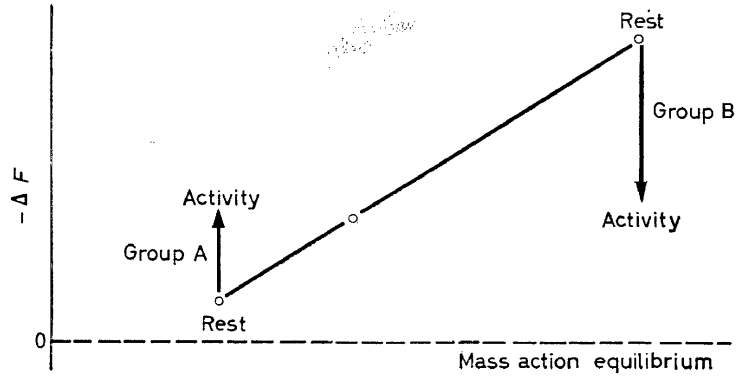


Figure 13. Relationship between flow equilibrium and mass action equilibrium at rest and during action. With increasing velocity in group A proportions are lifted off the mass action equilibrium, in group B they approach to it

Cori and his school[34, 35], have shown that, in this connection, a special meaning must be given to hexose phosphorylation. In our experiment also (*Figure 8*) this phenomenon is shown. In the pattern of metabolite content relatively large movements of fructose diphosphate can be seen and it must be borne in mind that the fructose-6-phosphate/fructose diphosphate quotient is several orders of magnitude greater than the value to be expected on the basis of equilibrium for phosphate transfer from ATP to fructose-6-phosphate[23]. It is easy to suppose, and it has in fact been stated by several research groups in recent years, that there exists between these metabolites a controlled non-equilibrium controlling glycolysis.

In fact the activity of enzyme phosphofructokinase is extraordinarily influenced by concentrations of bivalent cations and of adenosine triphosphate[36] (*cf.* also refs. 55 and 56). *Figure 14* shows experiments of this kind carried out with the enzyme from flight muscle of insect[23, 26]. Relatively small changes in the proportion of magnesium and adenosine triphosphate cause an increase of many orders of magnitude in the enzyme activity, from the flow velocity in the resting state to the flow velocity in flight.

It is possible that similar relationships occur also for other kinases in the course of energetic metabolism. It should be pointed out in this connection that in these metabolic passages there are reactions in the opposite direction in which phosphatases act and because of which the kinase action is wiped out (see *Figure 3*). Kinases and phosphatases can act together with the effect of an adenosine triphosphatase.

"Above" glucose-6-phosphate our experiment (*Figure 8*) indicates another

"floodgate". Whereas the proportion fructose-6-phosphate to glucose-6-phosphate corresponds to the equilibrium constant, the large increase in the level of both substances in activity leads us to the conclusion that there is another process of enzymatic activation[35, 37]. It is known from the research carried out for the first time by the St. Louis school that a kinase of special type has a part to play here[38, 39]. This is phosphorylase b kinase, an enzyme through whose action a second enzyme is activated. By action of adenosine triphosphate, and very probably with the participation of calcium and other factors of proteic nature, the slightly active phosphorylase b splits with

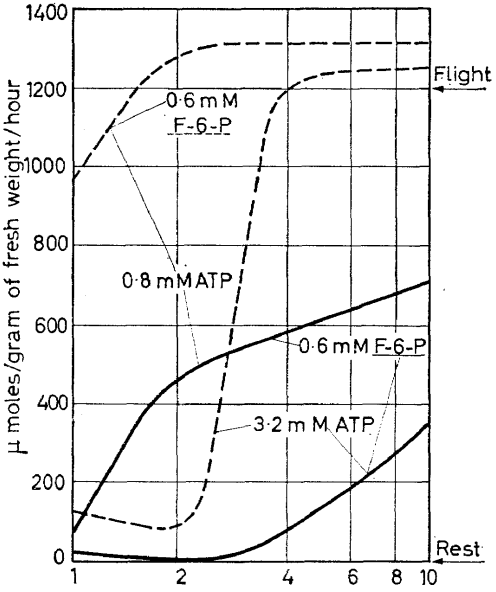


Figure 14. Influence of the levels of fructose-6-phosphate, ATP and Mg^{2+} on the work of fructose-6-phosphate kinase at constant concentrations
Abscissa: concentration of Mg^{2+} (mM)

phosphorylation into two molecules of very active phosphorylase a (point (6) in *Figure 3*). This process takes place when muscle is stimulated[34]; it results in the synthesis of phosphorylated hexoses from the glycogen reserve. The inverse transformation of the phosphorylase is brought about in a process going in the opposite direction through the action of a phosphatase.

The relationships between glycogen and phosphorylated hexoses is a particularly interesting sector in the organization plan of intermediate metabolism. The phosphorylase mechanism has, for example, been held responsible also for the effect of adrenalin on the liver, and of the adreno-corticotropic hormone on the adrenal cortex[40].

COMPARTMENTATION

Up till now the cell has been considered as a little bag full of a solution of enzymes and metabolites—a very rough approximation. To come nearer reality biochemists speak nowadays of compartmentation. This concept

from some points of view is ambiguous, it is, however, founded on an experimental basis and means a circumscribed "system" where a particularly strict co-ordination of chemical reactions exists. This concept was initially stated in this purely functional sense[37, 53, 54]. The causes of the phenomenon can, however, be very different: it can be a limited mobility of metabolites on the surface of a multi-enzyme system[17], an exclusive specificity towards a determined transfer metabolite by a group of enzymes[26], or even simply a separation through membranes with a specific permeability.

Each of these types of compartmentation has its own regulation mechanisms and therefore exerts its specific influences on the co-ordination of chemical processes in a living cell. An example of the first group is the control of cell respiration in mitochondrial membranes[3, 4]. For the second group we can cite the hydrogen transfer from the TPN-specific transport chains to the DPN-specific transport chains[41].

Concerning the third group, the subdivision of cell volume by membranes signifies that for a series of metabolites not only one concentration but several, and in certain cases as many concentrations as the numerable compartments, must be taken into consideration[27]. It must also be remembered that the sequences of the metabolic chains can pass through several compartments. The permeability of the membranes has therefore an important function in the co-ordination of chemical reactions in the cell.

Water too, in its double rôle as a vehicle of chemical mixtures and as a substance participating in many chemical reactions of the cell, must not be forgotten. It has been shown for example that thyroxine, in physiological concentrations, acts on the repartition of cell water between mitochondrial and extramitochondrial compartments[42–44].

In some of the examples the idea that the cell itself can be considered as a compartment in the association of tissue and organism has already appeared. Most metabolites are limited in their mobility to the cell. All the phosphorylated metabolites and many others belong to this group. How then are the chemical reactions of single cells co-ordinated to the performance of tissue and of entire organism? It will be necessary to concentrate our attention increasingly on the metabolites of intracellular fluids and blood plasma in view of this problem. Thus, there are good reasons for believing that lactic and pyruvic acid, as an oxido–reductive pair, co-ordinate the hydrogen pressure in the cells of a tissue and also of various tissues[26] (*Figure 5*, point 8, see also *Figure 10* and text).

PLASTICITY OF ENZYME PATTERNS

In the two large sectors of endocrine regulation concerning the organism as a whole, that is internal milieu and morphogenesis, a great difference can be seen from the point of view of action velocity. Most of the effects on the internal milieu occur in the space of minutes. Nevertheless, effects that set in slowly and last a long time, as for example the effect of thyroxine on basal metabolism, are known to exist. The effects on morphogenesis are long term ones.

These long term actions can be understood only by taking into consideration the plasticity of living matter which must, of course, include a plasticity in the co-ordination of cellular chemical reactions. Since this is something

fundamentally new, that is we have here an influence on the plan of organization, light must be thrown on this aspect by giving a final experiment.

The influence of thyroxine on the cell activity of enzymes was described more than a generation ago[45]. The most significant target organ of thyroxine action in warm-blooded animals is the liver. More-or-less all of its enzymatic levels are affected. Most variations do not, however, concern the order of magnitude. As has already been stated this means that most cell reactions undergo no substantial shift in the metabolite pattern. Recently, however, effects approaching a qualitative modification have been described[46]. Lardy and his colleagues have recently described a considerably stronger effect of the thyroid hormone on glycerophosphate oxidase (αGP-OX) than on other components of the respiratory chain[47]. If in *Figure 15* the activities of this enzyme after administration of thiouracil and after administration of thyroid powder are compared, a seventyfold difference in activity is seen[48].

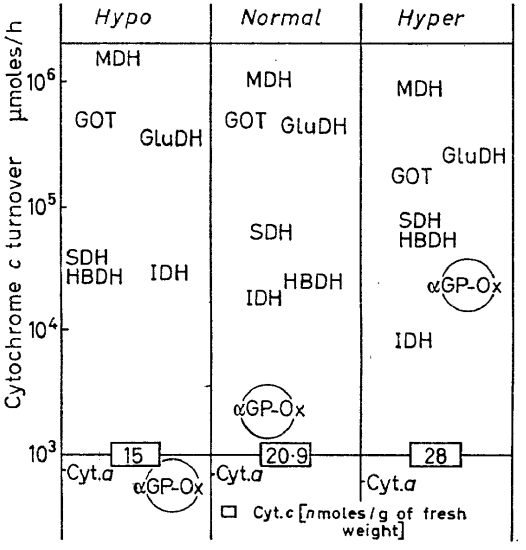


Figure 15. Patterns of mitochondrial enzyme activities (plotted as cytochrome *c* turnover on a logarithmic scale) in experimental hypo- and hyperthyroid and in the normal (euthyroid) state of rat liver. (MDH = malate-, GluDH = glutamate-, IDH = isocitrate-, SDH = succinate, HOBDH = β-hydroxybutyrate-, αGP-OX = α-glycerophosphate dehydrogenase, GOT = glutamateoxaloacetate transaminase); the absolute levels of cytochrome *c* are given at the base line

Very probably this is not a case of inhibition or acceleration of the existing enzyme activity, but a case of enzyme neoformation[47]. Metabolic regulation, therefore, occurs through neoformation or disappearance of an enzyme. The process takes time but it is an authentic control process at least so far as an enzyme level is regulated.

Can the metabolic action of thyroxin be explained with this experiment? Let us take a look at the pattern of hepatic metabolites in *Figure 16*. The system glycerophosphate/dihydroxyacetone-phosphate, system of oxido–reduction—found in the "normal" (euthyroid) condition at the same

reduction–oxidation potential as its sister systems (see *Figure 10* and text above) and even more in the hypothyroid state—has passed to a more oxidized state in thyrotoxicity[48]. Thus, the changes observed in the enzyme pattern are reflected by changes in the metabolite pattern which indicate an increased flow rate of hydrogen through the glycerophosphate system. These phenomena become even clearer if the organ is isolated from its various humoral interdependences by perfusion *in vitro*.

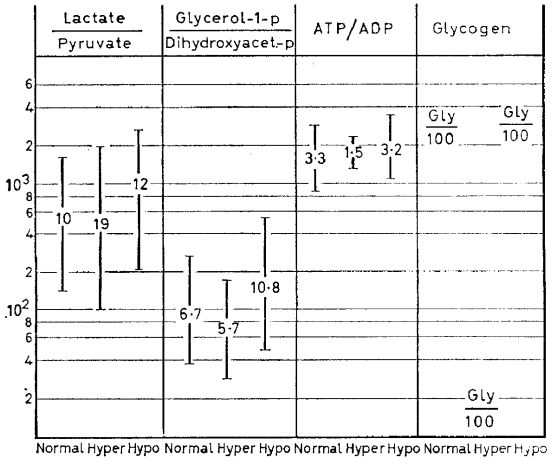


Figure 16. Liver metabolites under the influence of thyroid hormone; compare the similar presentation in *Figure 10*; state of livers as in *Figure 15*
Note minimal levels of glycogen and the low proportions [ATP/ADP], [glycerol-1-P/dihydroxyacetone-P] and [lactate/pyruvate] in the hyperthyroid liver[48]

The mitochondrial glycerophosphate oxidase has in recent years been thought to be connected with the hydrogen transfer from the glycolytic system to the respiratory chain, through the so-called glycerophosphate cycle (point (9) in *Figure 5*)[26, 41, 49, 50]. On this view an increase in the activity of glycerophosphate oxidase could effectively accelerate to a large extent the combustion of extramitochondrial available hydrogen, and consequently, produce a higher oxidation rate of glucose and lactate, just as it occurs in the long-term action of thyroxine on the liver.

It would be wrong to attribute all the metabolic effects of thyroid hormones on the body, or even only on the hepatic turnover, to the action just shown. I, however, believe, that we have here the key to a partial understanding of the effect of thyroxine on the increase of hepatic combustion. Therefore, these findings may demonstrate the importance of enzyme induction in the long term regulation of chemical systems. Similar processes are thought to play an important rôle alsó in drug action and drug tolerance[51, 52].

CONCLUSION

I gave at the beginning of this lecture part of what really belongs to the conclusion. After all, we might reflect on the fact that the greatest part of

metabolism is definitely automatically co-ordinated. Diseases which can be traced back to changes in the main metabolic pathways are clinically rare. From a practical point of view we can concentrate on only a few mechanisms. Analogy, a fundamental law of the living world, can help us in this, together with a knowledge of the plan of organization and the way in which the fundamental laws of chemistry and energetics act in it. Hence, the rather complicated considerations of the co-ordination of processes in the living cell today are not only of theoretical importance but they will also attain to practical interest in the future.

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