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ROLE OF STRUCTURE IN REGULATION OF CELLULAR ENZYMIC ACTIVITIES

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Abstract—Advances in enzymology have shown that a cell is not a mixture of enzymes, but a highly organized community where mitochondria, nucleus and endoplasmic reticulum are concerned in cellular activity. Quantities of enzymes appear to be of less importance in cell activity than correlations of enzymes with other factors such as structural barriers. Pyridinenucleotide-linked oxidations which take place in the extra-mitochondrial space of the cell proceed by way of extra-mitochondrial pyridinenucleotides. Experiments demonstrated that externally generated DPNH can pass the mitochondrial barrier to enter the respiratory chain. Fusion of enzymes to greater structural units would appear to give to the cell a level of organization between that of a single enzyme and the cellular entity.

TO THE modern cytologist the mitochondrion is the classical concept in subcellular organization. The electron microscopical and enzyme chemical complement of this organelle has been known in its main details for almost 10 years. Its ability to produce phosphate bond energy in the isolated state is well known to every biochemist.

The importance of mitochondrial research is, however, not restricted to a more or less advanced knowledge of the biochemistry and physiology of that particular subcellular component. It has also, for the first time, given the biologist a way of expressing himself in defined chemical terms and thus make himself understood by scientists working in other fields. For the biochemist, the realization that a cell is not a random mixture of enzymes but a highly organized community where components such as mitochondria, nucleus and endoplasmic reticulum all have their role in the maintenance of cellular activity, has brought a new concept to enzymology.

With the statement that a cell is able to do what its enzymic complement allows, as background, great efforts have been made during the post-war period to correlate the amount of enzymes in a cell with its physiological condition. For the cancer cell this has been particularly relevant, and a bulk of analytical data on the content of enzymes in tumours compared to normal cells have been collected. Also the activity of many enzymes in correlation to hormonal status has been a subject of intense study. In spite of the fact that many significant differences have been obtained, the enthusiasm for this type of research has gradually decreased and been replaced by the feeling that differences in the metabolic pattern of a cell must be correlated to factors other than quantities of enzymes.

Some examples of this phenomenon will be given below.

Perhaps the most serious problem a cell has to face is how to master the excess of enzymic activities it has at its disposal. How, for example, can it avoid combusting all substrate with the very high oxidizing power which can be attributed to a cell, and thus leave some of it as building blocks for new cellular material? Why, for example is a

tumour but not liver able to accumulate lactic acid? The cells from both tissues have, as we know, a considerable amount of lactic dehydrogenase at their disposal. For the solution of these problems the existence of structural barriers in the cell have to be considered.

As is well known, isolated mitochondria can oxidize all members of the citric acid cycle without the addition of any cofactors. Lactate, on the other hand, is not able to give rise to any respiration even if lactic dehydrogenase is added to the medium. Only after the addition of DPN can the mitochondria combust lactate. The dinucleotides present in the mitochondria cannot function as cofactors in the external lactic dehydrogenase system. This phenomenon, which is illustrated in Table 1 by an experiment

TABLE 1. REQUIREMENTS FOR THE OXIDATION OF LACTATE BY HEART-MUSCLE MITOCHONDRIA
(AFTER VON KORFF AND TWEDT[1])

Additions	μlO_2
None	2·4
Pyruvate	49·3
Lactate	4·0
Lactate + LDH*	8·1
Lactate + DPN†	13·5
Lactate + LDH + DPN	55·4

* LDH = lacticdehydrogenase.
† DPN = diphosphopyridinenucleotide.

quoted from Von Korff and Twedt[1], is also true of other extramitochondrial dehydrogenases. There is, therefore, reason to believe that those pyridinenucleotide-linked oxidations which take place in the extramitochondrial space of the cell, among them the oxidation involved in glycolysis, proceed also in the living cell by way of extramitochondrial pyridinenucleotides.

The acceptance of this statement immediately leads to a new problem: How are the extramitochondrially generated reduced pyridinenucleotides, e.g. the glycolytic DPNH, further oxidized by oxygen? The importance of this is obvious if we consider that in an aerobic cell, for each molecule of pyruvate formed from glucose, one molecule of extramitochondrial DPNH must be reoxidized by oxygen, provided that no accumulation of lactate takes place and no reductive synthesis of DPNH occurs parallel to the glycolysis. For a long time the main way known by which extramitochondrial DPNH could be oxidized aerobically by mitochondria has been that first described by Lehninger[2], which requires the addition of extramitochondrial cytochrome *c*.

An experiment by Von Korff and Twedt[1] demonstrates how hydrogen from non-mitochondrial substrates can be oxidized by way of the mitochondrial respiratory chain. This is, however, hardly a physiological mechanism, since cytochrome *c* is not known to occur in the ground cytoplasm of the cell.

Perhaps a more physiological experiment to demonstrate how externally generated DPNH can pass the mitochondrial barrier to enter the respiratory chain is to use a mitochondrial substrate, the oxidized form of which can be re-reduced by DPNH

formed in the ground cytoplasm. Zebe *et al.*[3] give an elegant example of this (Fig. 1). α-Glycerolphosphate is a mitochondrial substrate from the flight muscles of insects. This substrate is oxidized by means of a mitochondrial flavoenzyme to dihydroxyacetonephosphate which by means of an external enzyme and DPNH is re-reduced to glycerolphosphate. In this way glycerolphosphate becomes a cofactor for the trans-

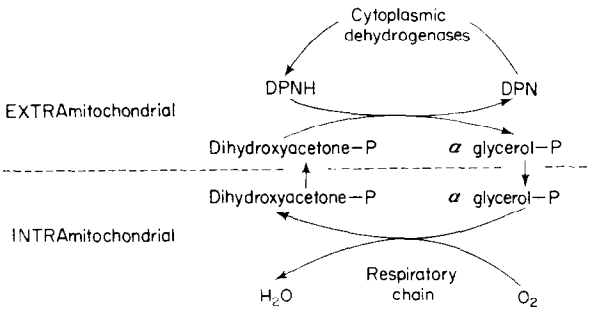

FIG. 1. Transport of hydrogen through mitochondrial barrier. (After Zebe, Delbrück and Bücher, *Biochem. Z.*, 1959).

portation of DPNH, formed in the ground cytoplasm, into the combustion oven of the mitochondrion.

The structure of this barrier between ground cytoplasm and mitochondria is a matter for discussion, but it seems certainly not to be a simple permeability problem.

An example of how structures can influence the activity of enzymes might be elucidated by the following experiment. To trap and conserve the energy formed

TABLE 2. ADSORPTION OF HEXOKINASE ON MITOCHONDRIA (AFTER SIEKEVITZ[5])

	Relative units
Added	100
Recovered in suspension after recentrifugation	89
Expected in pellet after recentrifugation	11
Found in pellet after recentrifugation	103

during mitochondrial oxidative phosphorylation, the addition of glucose and hexokinase is the best tool to use. This addition converts the ATP-formed into glucose-6-phosphate and ADP. In an attempt to calculate the amount of ATP present in the incubation medium from the differences in speed of formation and degradation, we obtained a much lower value for ATP than was expected.[4]

Siekevitz[5] working on the same system found that if the hexokinase is removed from the mitochondria by centrifugation, the minute amount of hexokinase which was adsorbed on the mitochondria, could after resuspension of the mitochondria in a fresh medium exert as high an activity as that originally added to the suspension. A considerable activation of hexokinase had evidently taken place. To explain this phenomenon is has been suggested that the hexokinase exerts its effect on the surface

of the mitochondrion. In this case a local high concentration of ATP might be responsible for the high enzymic activity. On the other hand, we know that only low concentrations of ATP are required for a high hexokinase activity in homogenized tissues. A further possibility is to apply the way of thinking introduced by McLaren and Bobcock[6]. These authors point out how surface structures can locally change the pH of a solution up to a whole unit, a phenomenon specially valid for cell surfaces. As hexokinase has its maximum activity at pH 8·5, and mitochondrial suspensions work at about pH 7·5, this offers a theoretical possibility for explaining the increased activity of the enzyme.

However these experiments are interpreted, they clearly demonstrate that the fusion of enzymes to greater structural units gives to the cell a level of organization between that of the single enzyme and the cellular entity. If this level of organization is going to be the background of hormonal control, physiological variation and cellular individuality will be a future subject of research.

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