COMPARABLE AND SPECIFIC PROPORTIONS IN THE MITOCHONDRIAL ENZYME ACTIVITY PATTERN

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Characterizing the significance of enzyme patterns, Greenstein stated: "Each of the enzymes behaves qualitatively in much the same way in all tissues. Quantitatively, however, the distribution of the enzymes varies..... and it is this quantitative distribution which constitutes the individual mosaic of each tissue". With the preceding (Pette et al., 1962) and present papers this view may now be extended in such a way that some important "stones of the mosaic" are not single enzymes but "constant-proportion groups" of enzymes.
The phenomenon of the extramitochondrial constant-proportion PTG-group (Bücher and Pette, 1961; Pette et al., 1962) has stimulated us to compare activity patterns of mitochondrial enzymes with respect to constant and specific proportions.

In the past years several authors (Hogeboom et al. 1948; Christie and Judah, 1953; Shepherd et al., 1954; Dickman and Speyer, 1954; Bücher and Klingenberg, 1958; Delbrück et al., 1959; Vogell et al., 1959; and others) have demonstrated that certain enzymes of the tricarboxylic acid cycle and of the metabolic segment of transamination are present in various tissues as intra- and extramitochondrial enzymes. In drawing up the mitochondrial activity patterns of different organs, the "multilocation" of these enzymes has been taken into account. However, the comparison of the different patterns dealt with in this paper has been strictly confined to mitochondrial enzymes.

Experiments

In the controlled procedure of fractionated extraction (Pette, 1962), soluble mitochondrial enzymes - malate dehydrogenase (MDH), glutamate dehydrogenase (GLUDH), TPN-specific isocitrate dehydrogenase (IDH), glutamate-oxaloacetate transaminase (GOT) - were extracted from 1 gram of tissue after the previous washing out of the extramitochondrial enzymes, and were tested under optimal conditions *in vitro* (Delbrück et al., 1959; Vogell et al., 1959). The activities of succinate dehydrogenase (SDH) and glycerol-1-P oxidase (GP-OX) were measured photometrically after extraction of the soluble extra- and intramitochondrial enzymes in the suspension of the insoluble and homogenized residue of 1 gram of tissue by a modification of the method of Slater and Bonner (Pette, Brosemer and Vogell, 1962). The maximum activities were evaluated from Lineweaver-Burk plots at different concentrations of ferricyanide. Pyruvate oxidase (PYR-OX) was determined amperometrically as the respiratory activity of isolated mitochondria with pyruvate as substrate in the presence of malate (Klingenberg and Slenczka, 1959). The activity was calculated by means of the cytochrome-factor (Schollmeyer and Klingenberg, 1962) as activity per gram of tissue.

Results

Fig. 1 summarizes the interrelations of mitochondrial enzyme activities in some of the tissues investigated. In this presentation, the absolute activities (activity/gr.tissue) have not been plotted, but rather their relation towards the content of cytochrome c is given by referring the different activities to the cellular level of cytochrome c. Thus the enzymic activities must be read as "cytochrome c-turnover" (dimension: 10^3/hour). The concentration of cytochrome a is expressed by its molar ratio to cytochrome c. By comparison of the various patterns it can be seen that the plotted activities and concentrations can be divided into groups of constant proportions (left-hand columns) and specific proportions (right-hand columns).

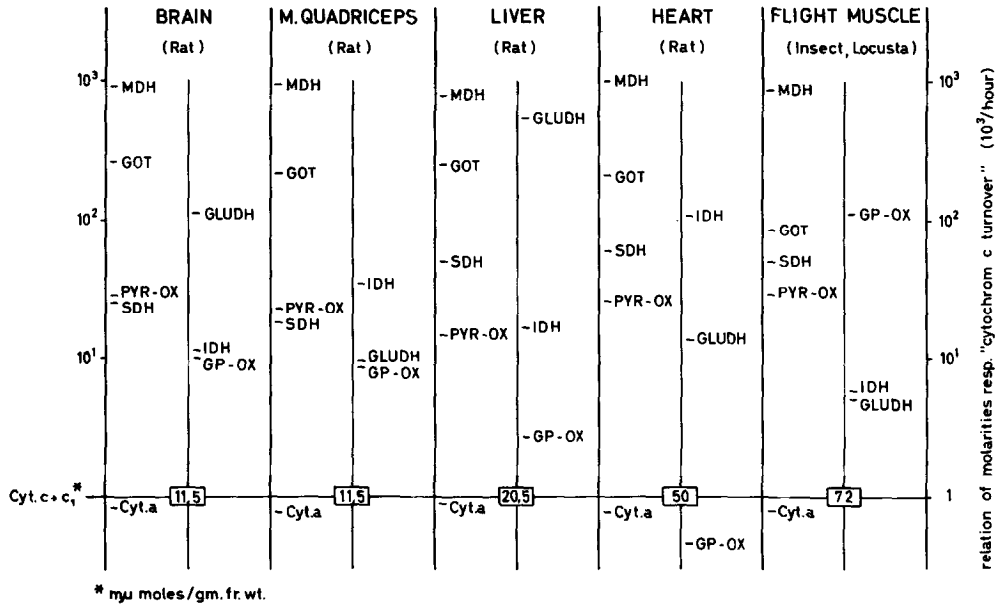

Figure 1

Constant proportions are found in the following segments of mitochondrial metabolism:

1) Respiratory chain. A striking constancy of the molar ratio of cytochrome a to cytochrome c is found in all the types of mitochondria investigated.
2) Pyruvate oxidation and tricarboxylic acid cycle. The activities of some of the enzymes tested prove to be related within the different mitochondria by their constant or nearly constant proportions. Thus the activity of MDH, expressed as "cytochrome c-turnover", is constant in the various patterns. Likewise, the activities of PYR-OX and SDH show only minor variations in different mitochondria when referred to the level of cytochrome c. The constancy of these proportions is stressed by the large degree of variation which is found in the range of the corresponding absolute activities.
3) In the metabolic segment of transamination the relative activity of GOT is largely constant and thus related not only to the concentration of the respiratory pigments but also to the activities of MDH, SDH and PYR-OX.

In contrast to these constant-proportion groups of enzymes, extremely varied proportions are found between the activities of those enzymes which are plotted in the right-hand columns. Thus IDH as a member of the tricarboxylic acid cycle, GLUDH as a key-enzyme of ammonia and amino acid metabolism, and GP-OX as the mitochondrial representative of the glycerol-1-P cycle show variations in the ratio of their activities to cytochrome c in the range of two orders of magnitude.

Discussion

Stoichiometric relations of the respiratory pigments in the order of 1 : 1 have been demonstrated by Chance in some types of mitochondria (cf. summary: Chance and Hess, 1959) and in our laboratory for a large variety of tissues (Schollmeyer and Klingenberg, 1962). The results dealt with in this paper confirm and complete these findings, although the ratio of cytochrome c to cytochrome a is in the range of 1.1 to 1.3 within the different mitochondria. In contrast to the extramitochondrial PTG-group (Bücher and Pette. 1961; Pette et al., 1962), the constant-proportion groups in mitochondria include enzymes from different segments of the metabolic network. Moreover, the phenomenon of constant proportions seems to be independent of the structural arrangement of these enzymes within the mitochondria and their different mode of binding to the mitochondrial matrix. In the case of constant-proportion groups of mitochondria, it becomes clear that not only the close interconnection of enzymes arranged one after another in the same segment of a metabolic chain is expressed by a constant ratio of activities, but also that the functional correlation of different enzymes placed at more distant points of the network may be revealed. Thus, for example, the constant ratio of the activity of GOT in regard to the constant-proportion groups of the tricarboxylic acid cycle and the respiratory chain indicates its importance as an auxiliary enzyme of the citric acid cycle, as has been suggested by Krebs and Lowenstein (1960).

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References

Bücher, Th. and M. Klingenberg, Angew.Chem. 70, 552 (1958)
Bücher, Th. and D. Pette, Proc.Intern.Congr.Biochem., Vth Congr., Moscow (1961)
Chance, B. and B. Hess, J.biol.Chem. 234, 2404 (1959)
Christie, G.S. and J.D. Judah, Proc.roy.Soc. B 141, 420(1953)
Delbrück, A., Zebe, E. and Th. Bücher, Biochem. Z. 331, 273 (1959)
Dickman, S.R. and J.S. Speyer, J.biol.Chem. 206, 67 (1954)
Greenstein, J.P., Biochemistry of Cancer, Chapt. VIII, New York (1954)
Hogeboom, G.H., Schneider, W.C. and G.E. Palade, J.biol.Chem. 172, 619 (1948)
Klingenberg, M. and W. Slenczka, Biochem.Z. 332, 486 (1959)
Krebs, H.A. and J.M. Lowenstein, in Metabolic Pathways, Vol. I, Academic Press, New York (1960)
Pette, D., Biochem.Z. (1962), in preparation
Pette, D., Brosemer, R. and W. Vogell, Biochem.Z. (1962) in preparation
Pette, D., Luh, W. and Th. Bücher, Biochem.Biophys.Res. Comm. 7, 419 (1962)
Schollmeyer, P. and M. Klingenberg, Biochem.Z. (1962), in press
Shepherd, J.A., Li, Y.W., Mason, E.E. and S.E. Ziffren, J.biol.Chem. 213, 405 (1955)
Slater, E.C. and W.D. Bonner jun., Biochem.J. 52, 185 (1952)
Vogell, W., Bishai, F.R., Bücher, Th., Klingenberg, M., Pette, D. and E. Zebe, Biochem.Z. 332, 81 (1959)