DPN LINKED ISOCITRATE DEHYDROGENASE IN NEAR CONSTANT PROPORTION TO THE RESPIRATORY CHAIN

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It has been demonstrated that enzymes belonging to the main pathways of metabolism are contained in various mitochondria in constant proportions between their activities (Pette, Klingenberg and Bücher, 1962). This concerns in particular the enzymes of substrate oxidation, such as the tricarboxylic acid cycle and the respiratory chain. Therefore, difficulties arose to the concept that isocitrate is normally oxidized via the TPN linked pathway, when the activity of the TPN linked isocitrate dehydrogenase as referred to the cytochrome content was found to vary considerably between different organs. In some cases such as insect flight muscle the activity of this enzyme is even too low to account for the normal turnover of the tricarboxylic acid cycle, whereas in other organs it is many times higher. On the basis of these striking divergencies the function of the TPN linked isocitrate dehydrogenase in the main path of the tricarboxylic acid cycle became improbable.

As shown in the preceding communication, a high activity of DPN specific isocitrate dehydrogenase (D-IDH) was found in all investigated mitochondria. Therefore this enzyme instead of the TPN specific one appears to be a possible member of the constant proportion group of enzyme activities pertaining to the substrate oxidation in the tricarboxylic acid cycle.

By comparing the activity of the D-IDH with the mitochondrial content of cytochrome a, as demonstrated in this paper,

a near constant relation of the D-IDH to the respiratory chain is observed. Thus, in the conjunction with the malate dehydrogenase, these two DPN linked soluble dehydrogenases of the tricarboxylic acid cycle form a "constant proportion group" with the respiratory chain.

Experimental procedure

The assay conditions for the DPN specific isocitrate dehydrogenase were briefly described in the previous paper; they are given in detail elsewhere (Goebell and Klingenberg, 1963 a). Malate dehydrogenase and TPN specific isocitrate dehydrogenase were assayed in the same extract as the DPN linked isocitrate dehydrogenase under standard conditions (Delbrück et al., 1959). Cytochrome a was determined in the intact mitochondria by absorption spectrophotometry (Schollmeyer and Klingenberg, 1962).

Results

The enzyme pattern in figure 1 gives the enzyme activity in reference to the cytochrome a content of the mitochondria. The "cytochrome turnover" thus obtained is plotted on a logarithmic scale. In relation to the respiratory chain concentration, the activity of D-IDH shows relatively small variations between the mitochondria of various organs. It can be regarded as near constant, when contrasted to the large variations of the relative activity of T-IDH between the different organs. The malate dehydrogenase (MDH), which was noted already earlier for its constant relation to the cytochrome content (Pette, Klingenberg and Bücher, 1962), shows still smaller variations than the D-IDH.

A compensation for the small variation of the D-IDH appears to be afforded by the activity of T-IDH. Thus, in organs with slightly lower relative activity of D-IDH, such as liver, heart and kidney an increased level of T-IDH is observed and conversely in brain and insect flight muscle the relative high activity of D-IDH is accompanied by a very low activity of T-IDH.

Discussion:

The enzymatic apparatus for the final substrate oxidation, namely the tricarboxylic acid cycle enzymes and the respi-

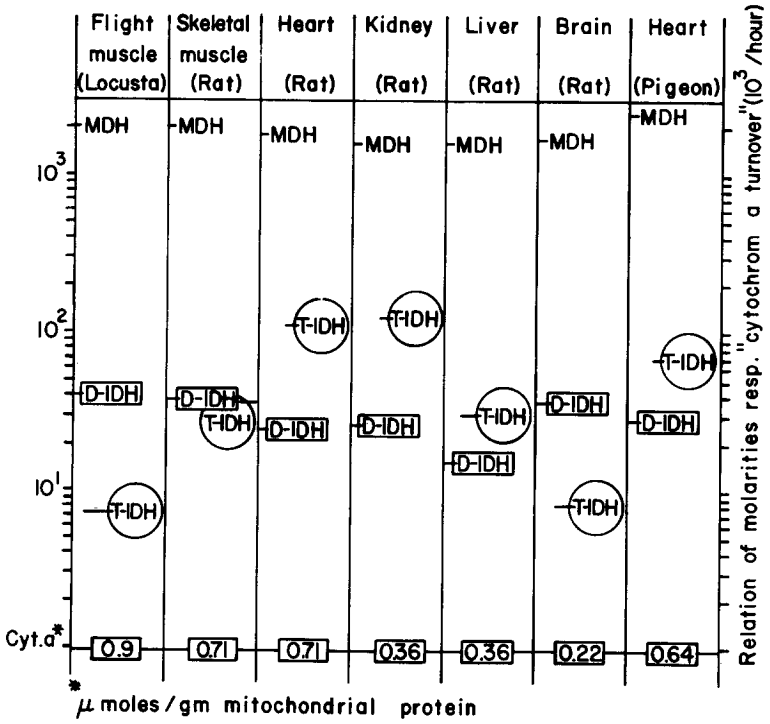

Figure 1

ratory chain, are generally acknowledged to belong to the standard equipment of mitochondria. Since quantitative data of enzyme activity and cytochrome contents have become available, this functional equipment can more precisely be defined by the finding of the constant proportional groups which should occur in both the tricarboxylic acid cycle and the respiratory chain. So far activities of soluble enzymes such as malate dehydrogenase fullfilled this concept best, but also particle bound enzymes such as succinate dehydrogenase appeared to fit into the picture (Pette, Klingenberg and Bücher, 1962).

The DPN specific isocitrate dehydrogenase, the only other DPN linked soluble dehydrogenase of the tricarboxylic acid cycle completes this pattern. This is the more remarkable, since in addition some mitochondria contain a high activity of the TPN linked enzyme. However, the results are in accordance with the concept that DPN is the coenzyme of the main

pathway of substrate oxidation, whereas TPN is related to more specific functions in the metabolism of the different organs (Bücher and Klingenberg, 1958). This view has been so far contradicted by the opinion that only the TPN linked pathway would count for the oxidation of isocitrate. Furthermore, this result agrees with the finding that also the DPN content of mitochondria displays a constant proportional behavior, in contrast to the TPN system (Klingenberg and Pette, 1962). Thus it is further substantiated that the constant proportion encompasses only the system of DPN linked pathways.

Therefore, the occurence of both a DPN and TPN specific enzyme for isocitrate dehydrogenation requires to assume a special role for each of these enzymes. The DPN linked pathway is part of the normal function of the tricarboxylic acid cycle in the complete oxidation of substrates for the generation of phosphate energy. The TPN linked pathway is coupled more to synthetic hydrogen consuming processes.

A mechanism for the regulation between both pathways obviously is given by the specific requirement of the D-IDH for ADP, in contrast to the T-IDH. These aspects will be discussed separately (Goebell and Klingenberg, 1963 b).

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