# PROPORTIONS OF MITOCHONDRIAL ENZYMES AND PYRIDINE NUCLEOTIDES

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In the preceding paper (Pette et al., 1962), interrelations of mitochondrial enzyme activities were discussed. Constant-proportion groups were demonstrated in three different segments of the metabolic network of mitochondria. Preliminary investigations (Klingenberg, 1960) suggested that the principle of constant proportions applies also to the relation between enzymes and coenzymes. On the basis of the more ramified data of enzyme activities in the intra- and extramitochondrial spaces as presented in the preceding communications (Pette et al., 1962), new results have been obtained by comparing the mitochondrial content of pyridine nucleotides and certain enzyme activities.

In figure 1 the mitochondrial contents of DPN and TPN (sum of oxidized and reduced forms) have been listed according to the ratio of their molar concentration to cytochrome c. As can be seen, the DPN content of various types of mitochondria fits the left-hand columns of constant proportions. Thus there are 6 to 12 molecules of DPN to one molecule of cytochrome c. Moreover, the constant ratio of cytochrome c and malate dehydrogenase (MDH), which represents in itself a constant-proportion group (Pette et al., 1962) consequently entails a constant ratio between these enzymes and the mitochondrial level of DPN.

In contrast to the above-stated constant proportion of DPN the mitochondrial contents of TPN have to be attributed to the specific proportions in the right-hand columns of figure 1. The level of TPN in regard to cytochrome c reveals large

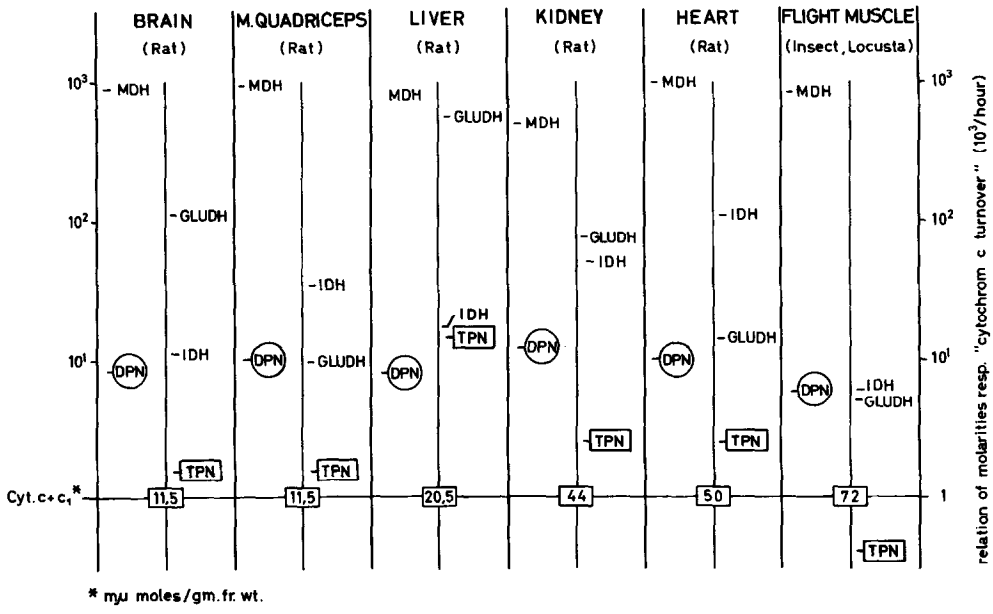


Figure 1

variations in the different types of mitochondria ranging from 0.4 in the insect flight muscle up to 14 in rat liver. On the other hand, despite these large variations a close relation exists between the different levels of TPN and the activities of isocitrate dehydrogenase (IDH) and especially of glutamate dehydrogenase (GLUDH). Among the various patterns one may differentiate two groups of organs which show a constant relation between the TPN content and the activity of a single dehydrogenase: in liver, brain, and kidney there is a nearly constant proportion between the level of TPN and the relatively high activity of GLUDH, while in the three different types of muscle - skeletal muscle and heart of the rat, flight muscle of the locust - a comparable relation is established by the TPN content and the activity of IDH. Nearly constant proportions, however, are found for all tissues when the sum of the activities of GLUDH and IDH is compared with the level of TPN. This finding led us to study the specificity of GLUDH in regard to DPN and TPN in the different organs. It has been found to be in correspondence in all cases. In the various extracts the activity of GLUDH in the presence of TPN amounted to 60 % of the activity measured

when DPN was used as coenzyme. With respect to the fact that the activities of GLUDH with DPN and TPN as tested *in vitro* in these different mitochondrial extracts are about equal, it seems advisable to discuss this finding in connection with the role of TPN as coenzyme of GLUDH *in vivo*.

It appears, that especially in the case of brain, kidney, and liver, glutamate dehydrogenase is primarily associated with the TPN system. With respect to the function of the TPN system as hydrogen donor for synthetic reactions (Krebs, 1954; Bücher and Klingenberg, 1958) and the relatively positive mid-potential of the glutamate-ketoglutarate redox-couple, the physiological action of GLUDH may be envisaged as the formation of glutamate and thus the feed-back of ammonia into the metabolism of the cell.

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