# CONSTANT-PROPORTION GROUPS OF MULTILOCATED ENZYMES*

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The phenomenon of constant-proportion groups in different metabolic pathways indicates a principle of quantitative coordination and control. So far, constant-proportion groups have been demonstrated in the intra- (i.m.) and extramitochondrial (e.m.) systems of energy-producing metabolism (Bücher and Pette, 1961; Pette et al., 1962; Klingenberg and Pette, 1962).In the case of malate dehydrogenase (MDH) and glutamate-oxaloacetate transaminase (GOT) it could be shown that only their i.m. activities reveal a constant ratio to the level of cytochrome c.With respect to the intracellular "multilocation" of MDH and GOT the question arises whether their e.m. activities are elements of an e.m. constant-proportion group and to what extent a relation may be found to exist between these two pairs of multilocated enzymes.

Correlations of enzyme activities within the mitochondria as cellular subunits are reflected by their relative activities to cytochrome c or mitochondrial protein. Their functional interrelations within the network of cell metabolism may, however, be read from their activities with reference to cellular protein or tissue weight. This has been done in figures 1 and 2, which summarize some of the results obtained. The activities of e.m. (left hand columns) and i.m. enzymes (right hand columns) extracted separately from 1 gram of tissue by fractionated extraction were tested under standardized conditions in vitro and plotted on a logarithmic

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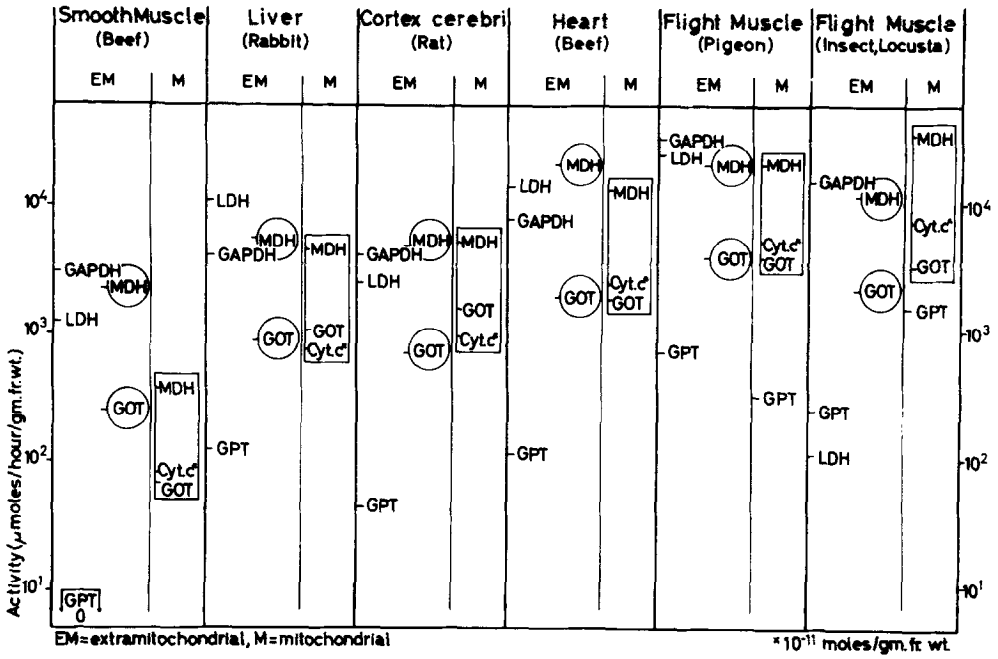


Figure 1

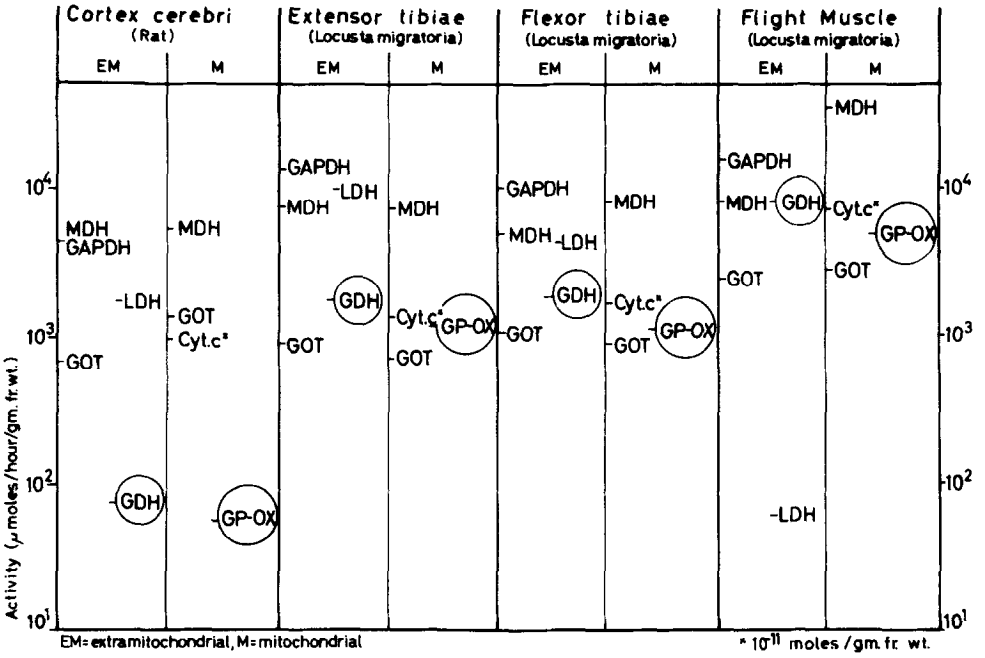


Figure 2

scale (Delbrück et al., 1959; Vogell et al., 1959; Pette et al., 1962).

The distribution of the activities of GOT and MDH between the i.m. and e.m. compartments differs from one tissue to another. For example, only 20 % of the total activity of MDH and 30 % of that of GOT is located within the mitochondria of smooth muscle, while 80 % and 60 % respectively are found within the mitochondria of the insect flight muscle. On the other hand, it can be stated that the ratio of the i.m. and e.m. activities of MDH is paralleled by the ratio established by the corresponding activities of GOT. Consequently, the e.m. activities of MDH and GOT are present in the different tissues in a comparable ratio ranging from 5 - 10. Thus the nearly constant proportion of the i.m. activities of MDH and GOT has its counterpart in the proportion established by the e.m. activities of the two enzymes. Moreover, a comparison of the different patterns confirms and augments our previous observation of a striking constancy in the mitochondrial activities of GOT and MDH in relation to the concentration of cytochrome c (Pette et al., 1962). In contrast, the ratio between the e.m. activities of GOT and MDH and cytochrome c is varied within the different tissues. However, in many of the tissues examined, a comparable proportion was found to exist between the e.m. activities of MDH and GOT and that of the constant-proportion phosphotriose-glycerate-phosphate group (PTG-group) which is represented here by the activity of glyceraldehydephosphate dehydrogenase - GAPDH - (Pette et al., 1962).

In order to examine the question whether the constant ratio established by the i.m. and e.m. activities of GOT and MDH primarily results from an inductive action of their coupling substrates, the activities of lactate dehydrogenase (LDH) and glutamate-pyruvate transaminase (GPT) have also been included in this comparison. However, the behaviour of these two enzymes is different in several respects. As shown by its extractability in different tissues, LDH is located exclusively within the e.m. compartment. This holds also for GPT in the majority of the tissues investigated. Nevertheless, in the leg muscles of the locust and in the flight muscle of the

locust and pigeon a multilocation of GPT could be demonstrated. Thus 30 % of the total activity of GPT in pigeon breast muscle and 85 % in the insect flight muscle are found within the mitochondrial fraction. Finally, in comparison with the constant proportion of MDH and GOT it can be seen that no relations of this kind exist for LDH and GPT. Likewise, neither LDH nor GPT shows a comparable ratio of its activity to that of the PTG-group as established in the parallel case of the e.m. activities of MDH and GOT in most of the tissues examined.

An example of correlated function of enzymes localized in separate cellular compartments is given by the e.m. (DPN-specific) glycerol-1-phosphate dehydrogenase (GDH) and the mitochondrial particulate glycerol-1-phosphate oxidase (GP-OX) in the operational system of the glycerol-1-phosphate cycle (Zebe et al., 1956; cf. Klingenberg and Bücher, 1960; Sacktor, 1961). Both enzymes linked across the mitochondrial membrane by their common pair of substrates show a constant ratio of their activities in different types of insect muscle (Vogell et al., 1959). Interestingly enough, the same ratio fits the pattern found in the cerebral cortex of the rat (fig.2) but not that in liver, cardiac and skeletal muscle.

The multilocation of GOT and MDH within the i.m. and e.m. compartment (Christie and Judah, 1953; Bücher and Klingenberg, 1958; Delbrück et al., 1959; and others) poses the question whether relations similar to that of GDH and GP-OX link the i.m. and e.m. activities of MDH and GOT (Bücher and Klingenberg, 1958). In this connection Borst (1961, 1962) recently discussed the possible mechanism of an aspartate-malate cycle. From this point of view, the constant-proportion pairs of MDH and GOT on each side of the mitochondrial membrane might be regarded as elements of a larger system and the phenomenon of the constant ratio of their activities might be explained. The interaction of both sets of GOT and MDH across the mitochondrial barrier would lead to a cyclic sequence of reactions connecting the e.m. and i.m. system of DPN/DPNH by moving aspartate and malate in opposite direction across the membrane, thus effecting hydrogen trans-

port by a circulation of intermediate metabolites. With respect to the possible function of MDH in this aspartate-malate cycle the inhibition of the i.m. MDH by oxaloacetate (Davies and Kun,1957; Bücher and Klingenberg,1958; Delbrück et al., 1959) and the inhibition of the e.m. MDH by malate (Siegel et al., 1960) appear to be of special interest.

So far, our interpretation of the data presented does not take into account the possible role of permeability barriers and the functional connection of GOT and MDH within the two cellular compartments has been deduced primarily from the constant proportion of their activities. However, from the results given in figure 1 it is clear that this constancy cannot be taken as a necessary consequence of the fact that the two enzymes are linked together by one of their substrates. As could be shown, no constant proportion is found in the parallel case of the activities of LDH and GPT.

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