STEADY STATE EQUILIBRIA OF SOME DPN-LINKED REACTIONS AND THE OXIDATION/REDUCTION STATE OF THE DPN/DPNH SYSTEM IN THE CYTOPLASMATIC COMPARTMENT OF LIVER CELLS IN VIVO

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In a previous communication (Honorst et al. 1959) it has been shown, that the ratios reductant / oxidant of three DPN-linked metabolite systems in the rat liver in vivo have the following relations:

1) $\{L/P\}^{+)} : \{G/D\} = 1.6\ K_{glyc.}/K_{lact.}$

1a) $\{M/O\} : \{G/D\} = 12.5\ K_{glyc.}/K_{mal.}$

K representing the mass action equilibrium constants of the corresponding DPN-coupled reactions$^{++)}$.

From this it has been concluded, that the steady state equilibria of these systems in vivo are very close to mass action equilibrium and therefore it should be possible to estimate the [DPN/DPNH] - ratio (i.e. the ratio of the free soluble DPN and DPNH) or in other words the true oxidation/reduction state of the DPN system

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+) Abbreviations and symbols:
{A} = level (overall content, "Gewebsgehalt") of substance A in the tissue; [A] = concentration of substance A in free solution. The relationship between the overall content and the concentration of metabolites in a defined cell compartment has been discussed in detail by Hohorst et al. 1959.
L/P = lactate / pyruvate , M/O = malate / oxaloacetate , G/D = glycerol-1-phosphate / dihydroxyacetonephosphate .

++) $\frac{[P] \times [DPNH]}{[L] \times [DPN]} \times [H^+] = K_{lact.} = 53 \times 10^{-13} M;$

$\frac{[D] \times [DPNH]}{[G] \times [DPN]} \times [H^+] = K_{glyc.} = 89 \times 10^{-13} M;$

$\frac{[O] \times [DPNH]}{[M] \times [DPN]} \times [H^+] = K_{mal.} = 9.8 \times 10^{-13} M$

(37°C; ionic strength 0.25 (Hohorst 1960)).

in the extramitochondrial compartment (C-compartment, Bücher et al. 1958) of liver cells.

This study has now been extended to livers in which L/P , G/D and M/O ratios had been changed. If under these conditions the relations 1) and 1a) were still valid, this would prove, that they really represent a functional relationship given by mass action equilibrium.

The change of the L/P , G/D and M/O ratios was achieved by subjecting liver tissue to short periods of ischaemia. The following technique was employed: Parts of rat liver (appr. 0.5 g) were cut off under ether narcosis (time 0). The excised tissue was left in the abdominal cavity, pressed at time t between two metal blocks previously cooled in liquid air and quickly immersed in liquid air. After tissue pulverization and perchloric acid extraction lactate, pyruvate, glycerol-1-phosphate, dihydroxyacetonephosphate, malate oxaloacetate and DPN^+ were determined in the neutral extracts by means of enzymatic tests (Hohorst et al. 1959). DPNH was measured in alkaline extracts according to Holzer et al. (1958).

The changes of the L/P , G/D and M/O ratios in relation to the duration of ischaemia are demonstrated in fig.1. The values plotted for time 0 are mean values as determined in non ischaemic livers with the technique published in a previous communication, Hohorst et al.(1959).

In fig.2 L/P and M/O ratios after different duration of ischaemia are plotted against the corresponding G/D values. The two regression lines are described by the equations:

2) $\{L/P\} = 2.0 \times \{G/D\} - 2.7$ 2a) $\{M/O\} = 12.7 \times \{G/D\} + 8.3$

The closeness of the correlation is demonstrated by the correlation coefficients $r = + 0.90$ (for 2)) and $r = + 0.86$ (for 2a)).

The slope of the upper line in Fig.2 is +12.7 and approxi-

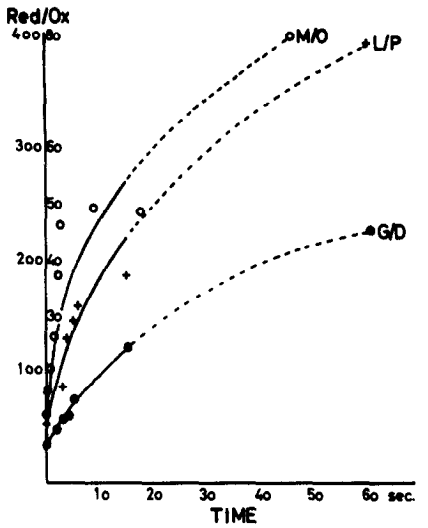

Fig.1. Ratios of reductant/oxidant in rat liver during ischaemia. Ordinate: (left) {M/O} ; (right) {L/P} and {G/D}

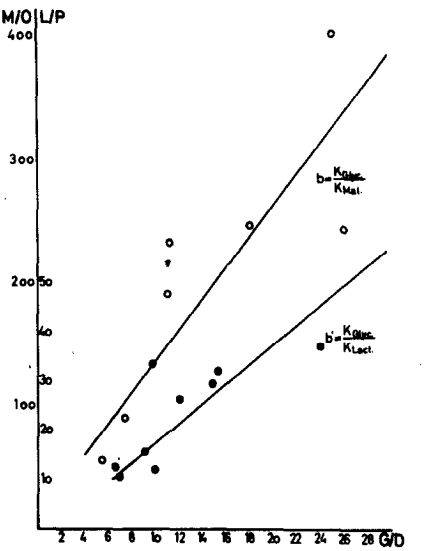

Fig.2. Regression lines and slope of {M/O}ooo and {L/P}●●● plotted against {G/D}. Ordinate: (left) M/O ; (right) L/P ; data from Fig.1.

mately equal to $K_{glyc.}/K_{mal.}$, that for the lower line is +2.0 and approximately equal to $K_{glyc.}/K_{lact.}$. (In neither case is the difference between the slope and the ratio of mass action constants significant). The slopes are therefore in accordance with the values to be expected for a mass action equilibrium. This proves, that the in vivo-relations 1) and 1a) as found in rat liver actually represent a functional connection given by mass action equilibrium. Even under the extreme conditions of ischaemia there are no measurable deviations of the steady state equilibria of these DPN-linked reactions from mass action equilibrium.

From fig.3 it can be seen, that the [DPN/DPNH] ratios for ischaemic tissue as calculated from the L/P -values according to

$$\frac{[DPN]}{[DPNH]} = \frac{[H^+]}{K_{lact.}} \times \frac{1}{\{L/P\}}$$ (assuming a constant pH-value of 7)

are considerably different from the {DPN/DPNH} -values obtained by direct analyses of DPN and DPNH (fig.3). {DPN/DPNH} is practi-

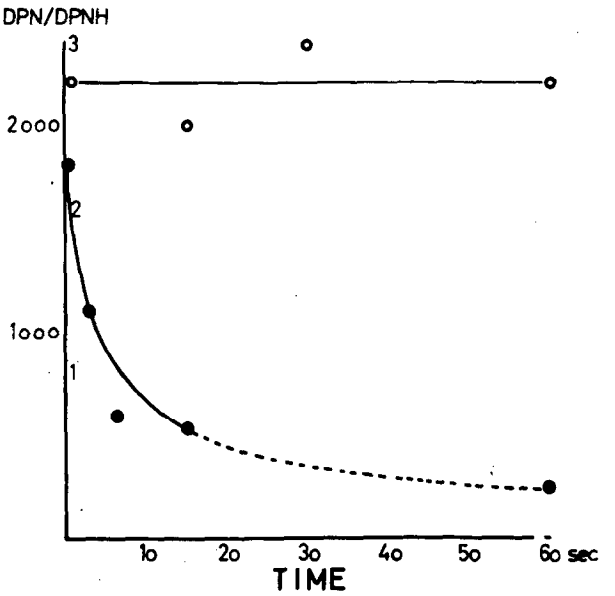

Fig.3. DPN/DPNH in liver during ischaemia.
Ordinate(left): [DPN/DPNH]●●● calculated from L/P -values in fig.1
Ordinate(right): {DPN/DPNH}ooo calculated from DPN and DPNH levels as determined by enzymatic test.

cally constant during a 60 second period of ischaemia, whereas [DPN/DPNH] decreases markedly indicating an accumulation of hydrogen in the extramitochondrial DPN system of the tissue.

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