THE OXIDATION/REDUCTION STATE OF THE EXTRAMITOCHONDRIAL DPN/DPNH SYSTEM IN RAT LIVER AND THE HORMONAL CONTROL OF SUBSTRATE LEVELS *IN VIVO*

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The reduction state of the DPN system in the extramitochondrial compartment (C-compartment, Bücher et al. 1958) of rat liver cells *in vivo* may be characterized by the ratios (Hohorst et al. 1959)

$$\{L/P\}^{+)} = 11 \pm 0.6, \qquad \{G/D\} = 6.7 \pm 0.6, \qquad \{M/O\} = 84 \pm 11$$

corresponding to an oxidation/reduction-potential of $E_h = -237$ mV[++)] and a $[DPN^+] / [DPNH]$ ratio of about 1.8×10^{3}[+++)]. The relative small statistical variation of the reductant/oxidant ratios indicates a high constancy of the oxidation/reduction state *in vivo*. On the other hand the DPN system has been shown to react very fast to alterations of the metabolic conditions such as short ischaemia of the tissue (Hohorst et al. 1961). This means, that the apparent stability of the oxidation/reduction state *in vivo* is not a static one, but bases on the steady state of reactions with high flow rates. Therefore the question was raised, how the oxidation/reduction state of the DPN system is controlled. Some data such as an elevation of L/P and G/D in alloxan-diabetic livers (Hohorst et al.1957 and Bücher 1960) lead to the suggestion, that hormones may play a part.

+) Abbreviations and symbols: Glycerol-1-P = L(-)α-glycerophosphate, DAP = dihydroxyacetonephosphate, OAA = oxaloacetate, DPN^+ and DPNH = oxidized and reduced diphosphopyridinenucleotide. $\{A\}$ = tissue level, $[A]$ = concentration of substance A (see Hohorst et al.1959). L/P = lactate / pyruvate , G/D = glycerol-1-P / DAP , M/O = malate / OAA.

++) on the basis of $E_{m7} = -204$ mV for L/P at 37°C (Hohorst 1960)

+++) assuming a pH-value of 7 in the C-compartment

Materials and methods: The animals used were male rats (Wistar), weight 150-200 g. For the adrenalectomy + fasted-series half-wild rats of a laboratory strain (Frankfurt/M) were employed[+]. All animals received the same diet[++]. Diabetes was produced by i.v.-injection of 50 mg alloxan/kg (5% alloxan-monohydrate in 0.001 N HCl); the blood- and urine-glucose (true glucose) was determined enzymatically after 8-12 days. The minimum blood-glucose- and urine-glucose-levels for a diabetic state were chosen as 200 and 2 000 mg% respectively. Animals in the fasted experiments received water ad lib. Bilateral adrenalectomy was performed under hexobarbital-narcosis; 0.9% NaCl solution was given ad lib. after the operation. For the adrenalectomy + diabetes-series alloxan-diabetes was induced first as above, followed by bilateral adrenalectomy of rats with a blood-glucose more than 350 mg% and urine-glucose more than 5 000 mg% (glucose determined twice over an 8-12 day interval between adrenalectomy and operation). Considerable decrease in blood-glucose occurred in each case after adrenalectomy. For the tissue preparation and determination of metabolites see Hohorst et al. (1959).

Results: As shown in fig.1 the L/P , G/D and M/O ratios in livers of alloxan-diabetic rats in vivo are 4-5times higher than those of normal controls. Since the sums of lactate + pyruvate and of malate + oxaloacetate are significant higher in the diabetes group, the observed decrease in pyruvate and OAA levels[+++] must be considered to be a consequence of the higher reduction state of the corresponding oxidation/reduction systems and not of a lack of C_3 and C_4 compounds in the liver. These alterations are reversible. 30 min.

+) This strain had less tendency to develop accessory adrenals.
++) Standard diet LAATZ, Euskirchen, Deutschland.
+++) These results are not in agreement with the data of Wieland et al. (1960) on oxaloacetate levels in diabetic livers.

after i.v.-injection of 20 I.U. insulin/kg[+] the values found were close to normal (series DI). Bilateral adrenalectomy effected an even greater normalisation (series DA) while intravenous injection of a glucocorticoid (60 mg prednisolone-succinate/kg[++]) on adrenalectomized diabetic animals led within 60 min. (15-60 min.) to similar alterations as found in the diabetic state (series DAC).

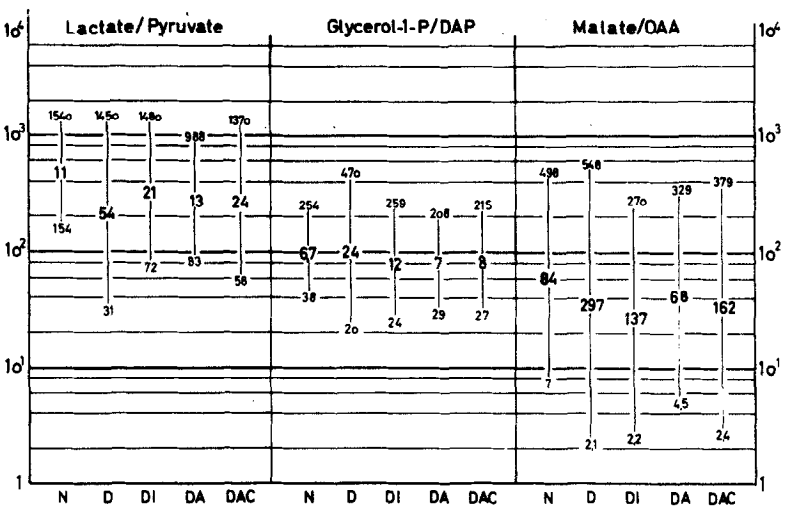

Fig.1. Tissue levels and reduction state of the systems: Lactate/pyruvate, glycerol-1-P/DAP and malate/OAA in rat liver. Series: N normal (17), D diabetic (13), DI diabetic+insulin (6), DA diabetes+adrenalectomy (3), DAC diab.+adrenalectomy+glucocorticoid (4). Numbers of animals in parentheses. Figures for the {L/P}, {G/D} and {M/O} ratios are placed between the levels of reductants (upper fig.) and oxidants (lower fig.).

The same type of changes was found in the fasting-experiments (see fig.2).

The increased reductant/oxidant ratios and malate[*] levels as found in the liver of 72[h] fasted rats were normalized two hours after

+) insulin Hoechst with 40 I.U./ml
++) Solu-decortin-H, E. Merck A.G., Darmstadt, Deutschland
*) OAA was not measured in this group.

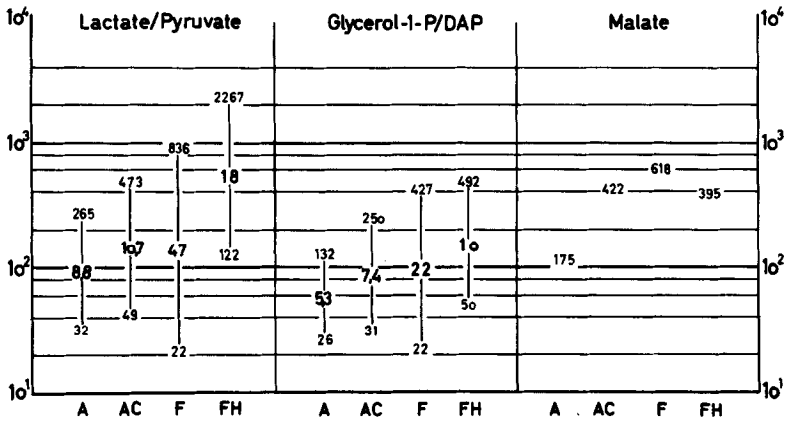

Fig.2. Tissue levels and reduction state of the systems lactate/pyruvate, glycerol-1-P/DAP and malate levels in the rat liver of fasting rats.
Series: A adrenalectomy+24^{h}-fasting(7), AC adrenalectomy+24^{h}-fasting+cortisol (8), F 72^{h} fasted(13), FH 72^{h} fasted+glucose (5); levels in muMoles/g$_{fresh\ weight}$. See also the legend to fig.1; for details see text.

i.v.-injection of 2 g glucose/kg. Bilateral adrenalectomy [+)] did not only inhibit the increase of the ratios and the malate level, but it also lowered these values compared to those in livers of normal non-fasting animals. On the other hand administration of glucocorticoids [++)] to adrenalectomized fasting rats was followed by an increase of the ratios and the malate level.

In fig.3 the M/O and L/P ratios measured in livers under different metabolic conditions are plotted against the corresponding G/D ratios. The regression line of the M/O values (upper curve) has a slope of +11.3 and is practically equal to the ratio of the mass action equilibrium constants $K_{glyc.}/K_{mal.}$ [*)] (theoretical value +9.1). The slope of the L/P -line (lower curve) is +2.3 and nearly equal to $K_{glyc.}/K_{lact.}$ (theoretical value +1.7). Both cur-

+) Food consumption of the adrenalectomized rats was already low before the fasting period, that fasting could not be extended for more than 24 hours.

++) 20-40 mg hydrocortisone per os.

*) $K_{glyc.}$, $K_{lact.}$, $K_{mal.}$ = mass action equilibrium constants for the DPN-linked reactions, see the preceding paper.

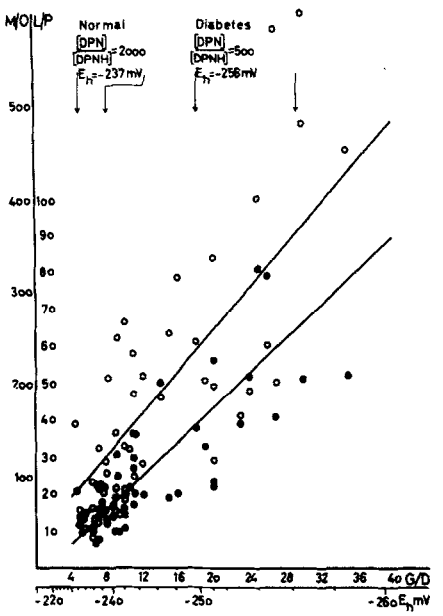

Fig.3. Regression lines of {M/O} and {L/P} plotted against {G/D}; data compiled from 56 experiments with rat livers in different metabolic state such as normal, fed, diabetes, insulin treated diabetes etc...(see fig.1)
Ordinate: (left) M/O ,ooo
(right) L/P .●●●
On the second abscissa: Oxidation/reduction potentials as calculated for the corresponding G/D values+). The [DPN/DPNH] ratios of the normal and the diabetic group are mean values evaluated for pH 7. The E_h-values represent the potential of the DPN system in the C-compartment.

ves are very similar to those, which have been published in the preceding communication (Hohorst et al. 1961). The high approximation of the slopes to the ratios of the mass action constants means, that even under abnormal metabolic conditions (diabetes, adrenalectomy, glucocorticoid-treatment etc.) the steady state equilibria of the three DPN-coupled systems lactate - pyruvate, glycerol-1-P - DAP and malate - OAA are very close to mass action equilibrium in vivo.

The results lead to the following conclusions:

1. The ratios {L/P}, {G/D} and {M/O} represent the oxidation/reduction state of the cytoplasmatic DPN system in liver cells in vivo on all metabolic conditions studied.
2. The oxidation/reduction state is influenced by hormones, glucocorticoids and insulin having antagonistic effects. Due to the very tight coupling of DPN reactions in the C-compartment

+) with E_{m7} = -211 mV for glycerol-1P/DAP, Hohorst 1960.

any action on the oxidation/reduction state will spread over the DPN system at a whole in this compartment.

3. In the liver of alloxan-diabetic rats the cytoplasmatic DPN system has been found to be more reduced. As a consequence of this pyruvate and oxaloacetate levels are lowered by a factor 3 - 5 in the diabetic liver.

4. The effects of insulin, corticosteroids and adrenalectomy on the metabolite state in diabetic and fasted liver lead to the assumption, that the alterations shown are due to a disturbance in the hormonal balance between insulin and glucocorticoids.

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