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2,4-Dinitrophenol-induced increase in ethanol and acetaldehyde oxidation in the perfused rat liver

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RECENT studies indicate that factors other than the amount (maximum activity) of alcohol dehydrogenase are also responsible for the regulation of hepatic alcohol elimination. It has been shown that the redox state of the cytosol (the free NADH/NAD ratio, which is in equilibrium with the lactate/pyruvate ratio) influences the elimination,[1] and it has been proposed that the level of acetaldehyde is also involved.[2,3] The rate of re-oxidation of the NADH formed during the metabolism of ethanol to acetaldehyde and then to acetate may be the major rate limiting factor.[3–5] Addition of a cytosolic NADH acceptor, such as pyruvate, stimulates ethanol oxidation.[6,7] Videla and Israel showed that 2,4-dinitrophenol (DNP), which uncouples oxidative phosphorylation and so accelerates NADH oxidation in the mitochondria, increases the rate of ethanol metabolism both *in vitro* and *in vivo*.[4,5]

We have examined further the mechanism of the DNP-induced stimulation of ethanol metabolism by means of a once-through liver perfusion system so that the oxidation of both ethanol and the acetaldehyde derived from it, the cytosolic redox state and the oxygen consumption could all be determined in the same liver

Livers were obtained during pentobarbital (Nembutal R., Abbot S.A., Brussels, Belgium) anaesthesia from male albino rats of Wistar origin, weighing 300–400 g and fed with standard laboratory diet and water *ad lib.* The once-through perfusion technique[3] was applied using Krebs-Ringer-bicarbonate solution equilibrated with $O_2 + CO_2$ (95:5) at 37°, and supplemented with 5·5 mM glucose, 1·5 mM L-lactate and 0·15 mM pyruvate. The flow rate was 40 ml per min. In preliminary studies 0·01, 0·02 and 0·05 mM DNP were passed consecutively through the same liver. Essentially the same effects were observed with each DNP concentration and the results obtained with 0·05 mM DNP are presented here as the average of four perfusions

The O_2 concentration in the effluent was measured with a Clark-type O_2 electrode. Ethanol and acetaldehyde were determined with a Perkin-Elmer F 40 gas chromatograph by head-space technique.[8] Metabolic rates were calculated as the product of the concentration difference between influent and effluent medium and the flow rate per wet weight of liver. The rate of acetaldehyde oxidation was obtained by subtracting the acetaldehyde output rate from the ethanol oxidation rate. Lactate and pyruvate in the medium were assayed enzymatically.[9] Enzymes and coenzymes were supplied by C. F. Boehringer (Mannheim, West Germany).

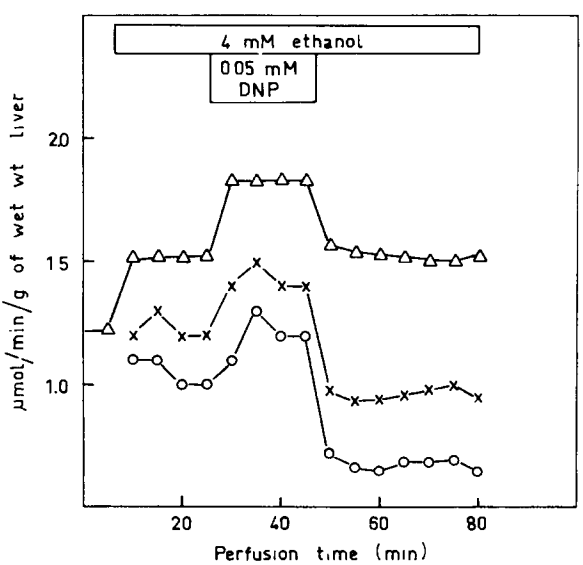

FIG. 1. Oxygen consumption (△), rate of ethanol (×) and acetaldehyde oxidation (○) in livers perfused without recirculation

There was a marked increase in the ethanol oxidation rate in the presence of 0·05 mM DNP (Fig. 1). The other two concentrations of DNP used in the preliminary perfusion series had a similar effect. The results agree with earlier findings in which DNP increased blood ethanol elimination in the dog[10] and, more recently, DNP was found to increase the ethanol oxidation rate in rat liver slices[4] and in the intact rat.[5]

The effect of DNP on the oxidation of the acetaldehyde formed from ethanol was very similar to that on the ethanol oxidation (Fig. 1) The increase in acetaldehyde and ethanol oxidation rates appear similar, but the decreased acetaldehyde output during DNP infusion (Fig. 2) clearly showed that the oxidation of acetaldehyde increased more than that of ethanol. Effects of DNP on acetaldehyde oxidation have not been reported before.

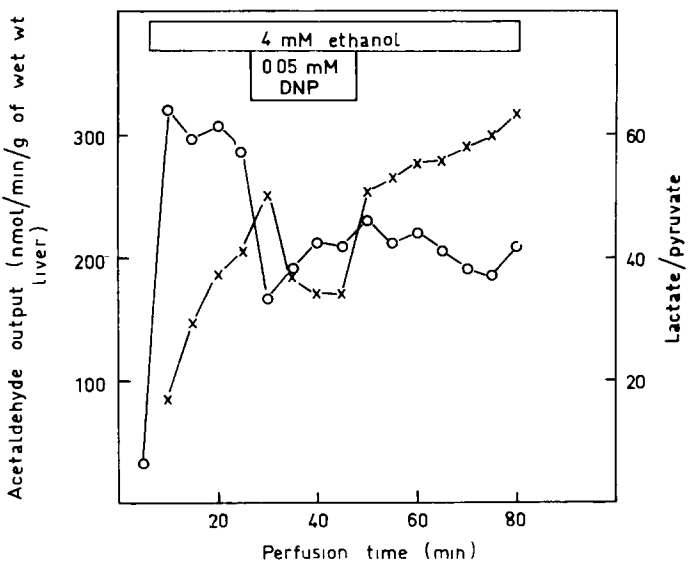

FIG. 2 Acetaldehyde output (×) and lactate/pyruvate ratio (○) in the effluent from livers perfused without recirculation.

Ethanol increased both the oxygen consumption and the lactate/pyruvate ratio in the perfusion medium (Figs. 1 and 2). DNP further stimulated the oxygen uptake but lowered the lactate/pyruvate ratio. Similar results were obtained in the preliminary perfusion series using three DNP concentrations (results not given here). The stimulatory effect of DNP on oxygen uptake during ethanol oxidation agrees with previous findings.[11] Videla and Israel reported that 0·1 mM DNP had no effect on the lactate/pyruvate ratio in liver slices incubated for 32 min with 10 mM ethanol and 10 mM glucose.[4] Vendsborg and Schambye found that after 30 min 0·6 mM DNP had increased the lactate/pyruvate ratio during ethanol metabolism in a re-circulating rat liver perfusion.[11]

In our experiments there was a transient decrease in the lactate/pyruvate ratio of the medium (Fig 2). which was accompanied by a similarly transient increase in the output of acetaldehyde. The increased acetaldehyde level may be a consequence of the decreased lactate/pyruvate ratio if it is assumed that the ethanol/acetaldehyde ratio is sensitive to the redox state.

The increase in NADH formation from ethanol and acetaldehyde oxidation roughly corresponds to the increase in oxygen uptake (Fig. 1). The somewhat stronger stimulation of acetaldehyde oxidation compared to that of ethanol supports at least partial intra-mitochondrial acetaldehyde oxidation if it is assumed that increased intra-mitochondrial NADH oxidation preferentially serves dehydrogenase reactions located in this cell compartment. The increase in the acetaldehyde oxidation rate is partly due to enhanced ethanol oxidation producing more acetaldehyde: the maximum rate of acetaldehyde oxidation in the liver is about 3–4 μmoles/min/g of liver wet wt,[3,12] which easily accommodates the acetaldehyde formed under these conditions.

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REFERENCES

1. M E. HILLBOM, *FEBS Lett.* **17,** 303 (1971)
2. H A KREBS, *Curr. Top. Cell Regul.* **1,** 45 (1969)
3. K O. LINDROS, R. VIHMA and O. A. FORSANDER, *Biochem. J.* **126,** 945 (1972).
4. L. VIDELA and Y. ISRAEL, *Biochem. J.* **118,** 275 (1970).
5. Y. ISRAEL, J. M. KHANNA and R. LIN, *Biochem. J.* **120,** 447 (1970)
6. M. E SMITH and H. W. NEWMAN, *J. biol. Chem.* **234,** 1544 (1959).
7. K. O. LINDROS, *Eur. J. Biochem.* **26,** 338 (1972).
8. C. J. P ERIKSSON, *Biochem. Pharmac.* **22,** 2283 (1973).
9. H. J. HOHORST, F. H. KREUTZ and T BÜCHER, *Biochem. Z.* **332,** 18 (1959)
10. P. L. EWING, *Q. Jl Stud Alcohol* **1,** 483 (1940).
11. P. B. VENDSBORG and P. SCHAMBYE, *Acta pharmac. tox.* **28,** 113 (1969).
12. L. MARJANEN, *Biochem. J.* **127,** 633 (1972).

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Apparent conversion of placental cytochrome P-420 to P-450 with *p*-chloromercuribenzoate*

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SPECTROPHOTOMETRIC and biochemical studies have indicated the presence of a carbon monoxide (CO)-binding cytochrome with an absorption maximum near 450 nm in both microsomal and mitochondrial subfractions of human placental homogenates.[1-5] A degraded form of this CO-complex has been designated cytochrome P-420 and, depending on the method of analysis, reportedly is observable as an absorption maximum in the 420–430 nm region in both human placental[1,4,5] and rat hepatic[6] microsomes. The degradation of hepatic microsomal cytochrome P-450 to the inactive P-420 form also is enhanced by sulfhydryl reagents such as *p*-chloromercuribenzoate (PCMB).[7] By contrast, polyols and reduced glutathione appear to stabilize the hepatic microsomal cytochrome.[7]

During investigations of placental CO-binding cytochromes,[4] we observed that low concentrations of PCMB seemed to reconvert cytochrome P-420 to cytochrome P-450 in human placental mitochondrial fractions. Therefore, this study was designed to investigate various factors such as temperature, protein concentration, ratios of P-420 to P-450 and stabilizing agents that might be required for this apparent conversion phenomenon and to draw comparisons between the effects of such factors on human placental mitochondrial vs rat hepatic microsomal CO-binding pigments.

Human term placentas were obtained from the delivery room of the University Hospital, University of Washington, and immediately transported to our laboratory where homogenization and differential centrifugation procedures were carried out as described by Juchau and Smuckler[8] (schedule 3). Hepatic microsomes were prepared from the livers of adult male Sprague–Dawley rats according to methods described by Mazel.[9] Cytochrome b_5 was prepared and purified from New Zealand rabbit liver microsomes according to the procedure of Strittmatter.[10]

Analyses of difference spectra were performed with a model DW-2 recording spectrophotometer (American Instrument Co.). Each spectrum was calibrated against the absorption maximum of reduced

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