N-demethylation of (+) benzphetamine decreased 70 per cent and the *p*-hydroxylation of aniline decreased 50 per cent in 96 hr of storage. However, cytochrome b_5 increased about 10 per cent, while cytochrome P-450 decreased 10 per cent in 96 hr. Levin *et al.*[3] suggest that when liver microsomal preparations must be stored for longer than 1 day the microsomes should be stored as a pellet at $-15°$ rather than as a suspension or as a lyophilized powder. Leber and Bousquet[6] found that an acetone powder preparation of rat hepatic microsomes was active in the oxidative metabolism of hexobarbital, aminopyrine, *p*-nitroanisole and the reductive metabolism of *p*-nitrobenzoic acid. Kuntzman *et al.*[2] reported the effects of storage on hepatic microsomes of excised whole liver which was frozen overnight.[2] Jondorf and Donahue[4] reported that the decline of microsomal *N*-demethylation of aminopyrine can be prevented when the rat carcass or excised livers are stored at 0–4° and assayed within 6 hr. Wade *et al.*[7] observed that rat and rabbit microsomal cytochrome P-450 is more stable when stored as the microsomal pellet at $-15°$ than when stored either as the pellet at 0° or as a suspension at $-15°$ or 0°. While commenting that sample storage at $-15°$ should allow maintenance of microsomes for at least 8 days after re-ultracentrifugation, without significant loss of cytochrome P-450 content, these authors did not determine the possible changes in enzymic activity of microsomes that may have occurred during storage.

Data presented in this paper indicate that microsomal suspensions, when quick-frozen with liquid nitrogen and stored at $-85°$, do not display loss of stability as reported by other workers. No significant losses of enzymic activities were observed at the end of a 1-week storage when compared to those of fresh microsomes, except a gradual decrease in NADPH oxidase activity.

Acknowledgement—The authors gratefully acknowledge that this investigation was supported by United States Public Health Service Grant CA-034808 from the National Cancer Institute.

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Biochemical Pharmacology, Vol. 23, pp. 2234–2337. Pergamon Press, 1974. Printed in Great Britain.

Activation of ethanol metabolism by 2,4-dinitrophenol in the isolated perfused rat liver

(*Received* 29 *October* 1973; *accepted* 21 *January* 1974)

IT HAS PREVIOUSLY been proposed that the mitochondrial reoxidation of NADH normally constitutes the rate-limiting step of alcohol metabolism in the liver, because uncoupling agents are able to increase the rate of alcohol metabolism in liver slices[1] and in the intact animal.[2,3] It can be argued, however, that

the rate of alcohol metabolism in liver slices is less than one-third of that found in the intact animal, and that different metabolic reactions may be involved in the two situations. Although the rate of alcohol metabolism is also increased by 2,4-dinitrophenol (DNP) *in vivo*, it is difficult to ascertain that the effect of DNP is exclusively in the liver. We have, therefore, studied the effect of DNP on the rate of ethanol metabolism in the isolated perfused rat liver in which ethanol is metabolized at a rate which is 60–70 per cent of that found *in vivo*.

Male Wistar rats (High Oak Farm, Goodwood, Ontario) weighing 275–300 g were used for these experiments. The liver was removed under pentobarbital (60 mg/kg) anesthesia (Nembutal Veterinary, Abbott, 60 mg/ml), essentially as described by Fisher and Kerly[4] except that the hepatic artery was neither ligated nor cut before initiating portal pre-perfusion, so that at no time was the liver entirely deprived of oxygen. Pre-perfusion was carried out with Krebs–Henseleit–Ringer solution, pH 7·4 (117·78 mM NaCl, 4·69 mM KCl, 2·48 mM $CaCl_2$, 1·16 mM KH_2PO_4, 1·18 mM $MgSO_4 . 7 H_2O$, 24·3 mM $NaHCO_3$, 9·94 mM glucose), kept at 37°. A mixture of 95% O_2:5% CO_2 was used to saturate all solutions during pre-perfusion and perfusion. The liver was then transferred from the animal to the perfusion chamber. The perfusion apparatus was basically that described by Bloxam,[5] modified for use as an open flow system. Perfusion rate was 5–6 ml/min/g of liver wet weight at a constant portal venous pressure of 14 cm of water. The liver was completely covered with polivinylidene film (Saran, Dow Chemical Co.) to prevent oxygen exchange with the gas system saturating the chamber. Livers were first perfused with 1 litre of the Ringer solution and thereafter with the same solution containing 3 mM ethanol.

DNP was infused with a pump at a constant rate through the open end of the Fisher portal vein cannula. When oxygen consumption was measured, the effluent was passed through a Clark oxygen electrode (Yellow Springs Inst.) mounted in a plexiglass flow-through chamber. Sampling started after about 400 ml Ringer–ethanol solution had been passed through the liver. Residual ethanol concentration in the effluents was determined every min by the alcohol dehydrogenase method.[6] Ethanol metabolism and oxygen uptake were calculated from the pre- and post-perfusion concentrations and from the flow rates, which were measured concurrently.

Figure 1 shows a complete experiment from a series of four experiments in which the rate of alcohol metabolism and the rate of oxygen consumption were measured simultaneously in livers from fed animals. Data showing the maximal activatory effect of DNP for the other experiments are presented in Table 1. The basal rate of ethanol metabolism in these preparations was of the order of 1·5 to 2·0 μmoles/g liver/min. This is in good agreement with values reported by other workers using the perfused liver technique, under similar conditions.[7,8] Oxygen consumption rates were in the upper part of the range reported by other workers.[5,7,8] DNP in concentrations up to 60 μM increased both the rate of oxygen consumption and the rate of ethanol metabolism. Higher concentrations of DNP further increased the rate of oxygen consumption but had no effect on that of ethanol metabolism or even reduced it. In general, we have

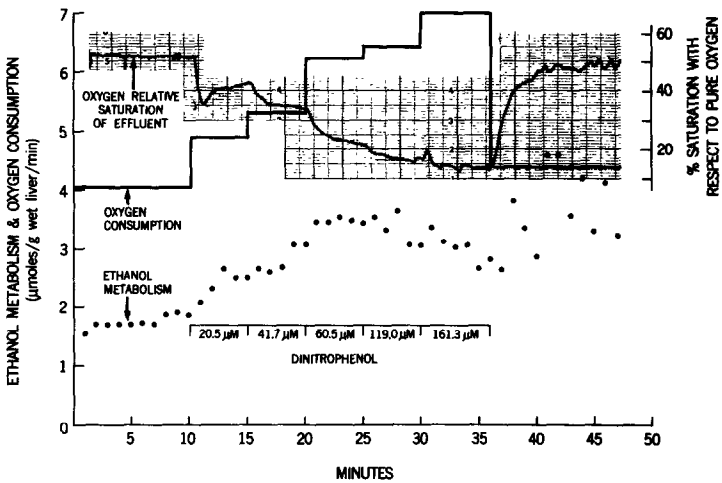

FIG. 1. Effect of dinitrophenol on the rates of ethanol metabolism and oxygen consumption in the isolated perfused rat liver. Representative experiment in animals fed *ad lib*. Oxygen consumption and ethanol metabolism were calculated at 1-min intervals from the pre- and post-perfusion concentrations and the perfusion flow rate. The original continuous recording shows the oxygen tension of the effluent. The inflow perfusate was saturated with 95% O_2:5% CO_2.

TABLE 1. ACTIVATION BY DINITROPHENOL ON THE RATE OF OXYGEN CONSUMPTION AND ETHANOL METABOLISM IN PERFUSED LIVERS*

Expt.	Concentration of dinitrophenol (μM)	Increase in oxygen consumption (μmoles/g liver/min)	Increase in ethanol metabolism (μmoles/g liver/min)
1	60·5	2·22	1·78
2	38·9	1·42	1·18
3	39·8	1·59	2·48
4	41·6	1·54	0·57
Mean ± S. E.	45·2 ± 5·13	1·69 ± 0·18	1·50 ± 0·41

* Increases correspond to the difference between the basal rates in the absence of dinitrophenol and those in its presence.

found that concentrations of DNP higher than 100 μM produce transient effects on ethanol metabolism, in some cases yielding values below those obtained in the absence of DNP. This, however, does not occur with the rate of oxygen consumption, which always remains increased. Similar observations have been reported by Williamson *et al.*[9] for the metabolism of xylitol in the perfused rat liver. For ethanol this could conceivably occur if, in a maximally uncoupled state, cytoplasmic-reducing equivalents fail to enter the mitochondria. It has been reported that the transport of malate—a constituent of the malate–aspartate shuttle—into the mitochondria is inhibited by uncoupling agents.[10] A reduction in the translocation of reducing equivalents from the cytosol into the mitochondria by high concentrations of DNP would also be in line with data by Vendsborg and Schambye[11] who found that, in perfused livers in the presence of ethanol, high concentrations of DNP (667 μM) markedly reduced the intramitochondrial β-hydroxybutyrate/acetoacetate ratio, while increasing the lactate/pyruvate ratio in the cytosol. A further factor tending to raise the lactate/pyruvate would be the effect of DNP on ATP/ADP × Pi levels.[12]

An unexpected finding was that, after rather long perfusions in the presence of DNP (more than 5 min), removal of the uncoupler does not reverse the rate of alcohol metabolism to basal values, while doing so to the rate of oxygen consumption. Under these conditions, about 80 per cent of the oxygen utilized by the tissue would be used to convert ethanol into acetate, in contrast with about 45 per cent in control

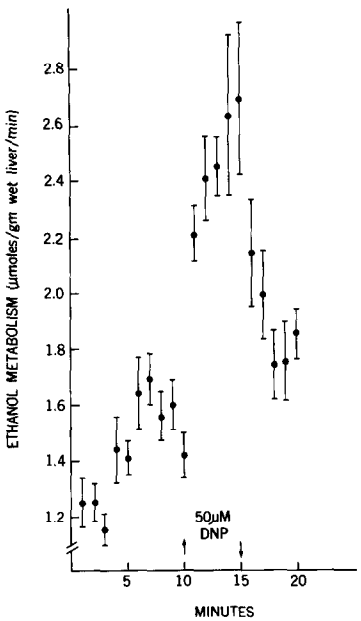

FIG. 2. Activatory effect of dinitrophenol on the rate of ethanol metabolism in perfused livers. The values represent the mean ± standard errors for five experiments. The animals were fasted overnight prior to sacrifice.

livers. An alternative mechanism would be an oxygen-independent ethanol utilization, in which the NADH produced by ethanol metabolism would be reoxidized by oxidized compounds that could accumulate during the perfusion with DNP. Further work is necessary to fully understand this effect.

Figure 2 shows the results of a group of five experiments done in order to study the effect of DNP in animals fasted overnight, and the reversibility of the effect after a short infusion of the uncoupler. DNP was infused at 50 ± 2 μM. The overnight period of fasting (18 hr, water *ad lib.*) did not alter the baseline metabolism of ethanol when compared to fed animals. This is in agreement with data by other workers[13] showing that, while a 48-hr fasting period markedly reduces the rate of ethanol metabolism *in vivo*, no changes can be observed after a fasting period of 18 hr. DNP increased the rate of alcohol metabolism by about 100 per cent, in agreement with the observations in fed animals and also in line with previous findings in liver slices.[1] The effect of DNP was rapidly reversed under these conditions, in which the liver was exposed for only 5 min to the uncoupler.

In conclusion, the data presented here strongly indicate that the rate of ethanol metabolism is limited by the mitochondrial oxidative capacity.

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Biochemical Pharmacology, Vol. 23, pp. 2337–2339. Pergamon Press, 1974. Printed in Great Britain.

Effect of estradiol on lipolysis and adenosine 3′,5′-monophosphate accumulation in isolated rat adipocytes

(*Received* 10 *October* 1973; *accepted* 31 *January* 1974)

IN RECENT years a considerable amount of evidence has accumulated which demonstrates some influence of estrogens on lipid metabolism. Experiments which were performed mainly after chronic administration of the hormone (14 days and longer) showed an elevation of serum triglycerides[1,2] and total glycerol[3] as well as changes in circulating lipid composition.[3,4] Little is known, however, about the acute effects of estrogens on the metabolism of fat and whether there are any direct estrogen-effects on adipose tissue. In order to get some information on a possible estrogen-induced lipolytic action eventually mediated by cyclic 3′,5′-AMP accumulation we have investigated the influence of estrogens on lipolytic systems *in vivo*