1% agarose in 50mm-barbiturate buffer, pH 8.6; sample troughs, 10mm×2mm, are cut 2cm from the bottom and a 'window' for the antibody-containing gel is cut 1.5cm above the troughs. During electrophoresis the antibody moves towards the troughs, and this gap ensures that it cannot enter the tissue and cause precipitation *in situ*. Samples of intima (area 0.25–1.0cm²) are finely minced with scissors, folded in squares of lens-paper and pressed into three troughs, and 10μl of ultracentrifugally standardized serum is placed in the fourth on filter paper. The troughs are filled with agarose and the plates run inverted at 3V/cm for 40h. The tissue 'parcels' are removed for further analysis, and for β-lipoprotein the plates are washed in water for 3h before being dried and stained overnight in Oil Red O. The areas of the stained peaks are measured and the β-lipoprotein is calculated from the standards. The area is proportional to the volume of standard serum over the range 5–20μl (see Clarke & Freeman, 1966).

Tissue dry weights were 0.4–6.5mg, and in 25 samples comprising normal intima, fatty streaks and fibrous thickenings the concentrations of β-lipoprotein cholesterol lie within the ranges found previously (Smith & Slater, 1970).

The relationship between β-lipoprotein and other plasma proteins can also be examined; in preliminary experiments with five samples the β-lipoprotein/albumin ratio was determined in terms of μl of standard serum/100mg dry wt. of tissue. In all samples β-lipoprotein exceeded albumin: in the upper half of normal intima by 6.1- and 7.5-fold, in the underlying layers by 2.3- and 1.9-fold and in an early fibrous plaque by more than 10-fold, which strongly suggests that it is being preferentially retained.

This work was supported by a grant from the British Heart Foundation.

Clarke, H. G. M. & Freeman, T. (1966). In *Protides of the Biological Fluids*, vol. 14, p. 503. Ed. by Peeters, H. Amsterdam: Elsevier Publishing Co.

Fahey, J. L. & McKelvey, E. (1965). *J. Immun.* **94**, 84.

Ressler, N. (1960). *Clinica chim. Acta*, **5**, 359.

Smith, E. B. & Slater, R. S. (1970). *Atherosclerosis*, **11**, 417.

The Action of Fructose on Ethanol Metabolism by Intact Isolated Liver Cells

By MICHAEL N. BERRY. (*Department of Pathology, University of California, San Francisco, Calif. 94122, U.S.A.*)

Morphologically intact isolated parenchymal cells from rat liver (Berry & Friend, 1969) were used to examine the hepatic metabolism of ethanol. Under

all experimental conditions over 90% of the ethanol removed could be accounted for by acetate formation. Cells from fed or starved animals, incubated with ethanol alone, formed acetate at a mean rate of $0.77 \pm 0.02 \mu\text{mol/min per g wet wt.}$, and this was increased to $2.24 \pm 0.07 \mu\text{mol/min per g wet wt.}$ if 10mm-fructose or 10mm-D-glyceraldehyde was also added. In the presence of fructose the accumulation of sorbitol, glycerol and α-glycerophosphate accounted for only about 25% of the reducing equivalents arising during ethanol oxidation. Hence fructose or its metabolites promoted the transfer to oxygen of hydrogen arising during ethanol metabolism.

The fructose stimulation of ethanol oxidation by the isolated cells was not affected by the glycolytic inhibitors iodoacetate or fluoride, or by the presence of the mitochondrial substrates 10mm-3-hydroxybutyrate or 2mm-palmitate, but was inhibited by over 90% by rotenone. The fructose effect was completely abolished by 0.25mm-pyrazole, an alcohol dehydrogenase inhibitor (Theorell & Yonetani, 1963), implying an essential role for this enzyme in fructose-enhanced ethanol oxidation. However, in view of the suggestion that an NADPH-dependent microsomal pathway might be of importance for ethanol metabolism (Lieber & DeCarli, 1968), the effects of various drugs known to be metabolized by the endoplasmic-reticulum hydroxylase system were examined. Acetanilide, aminopyrine, aniline or antipyrine had no effect on the basal rate of ethanol oxidation, but acetanilide and aminopyrine inhibited the fructose stimulation of ethanol oxidation by 45% and antipyrine or aniline decreased it by 85%. These drugs had no effect on cell adenine nucleotide concentrations or on the metabolism of fructose when added in the absence of ethanol. Moreover, in contrast with pyrazole they did not correct the greatly increased lactate/pyruvate concentration ratio induced by ethanol.

It is concluded that the rate-limiting step in ethanol oxidation by isolated liver cells is the transfer to oxygen of reducing equivalents arising in the alcohol dehydrogenase-catalysed oxidation of ethanol. This transfer appears to be promoted by fructose or its metabolites and may be influenced by microsomal electron transport.

This work was supported by U.S. Public Health Service Grant AM-12284.

Berry, M. N. & Friend, D. S. (1969). *J. Cell Biol.* **43**, 506.
Lieber, C. S. & DeCarli, L. M. (1968). *Science, N.Y.*, **162**, 917.

Theorell, H. & Yonetani, T. (1963). *Biochem. Z.* **338**, 537.