6. G. J. BREWER and J. W. EATON, *Science, N.Y.* **171,** 1205 (1971).
7. J. W. EATON, G. J. BREWER and R. F. GROVER, *J. Lab. clin. Med.* **73,** 603 (1969).
8. R. E. KRAVATH, A. S. AHARON, G. ABAL and L. FINBERG, *Pediatrics* **46,** 267 (1970).
9. P. TRINDER, *Biochem. J.* **57,** 301 (1954).
10. R. W. WINTERS, J. S. WHITE, M. C. HUGHES and N. K. ORDWAY, *Pediatrics* **23,** 260 (1959).
11. P. ASTRUP, M. RÖRTH and C. THORSHAUGE, *Scand. J. clin. Lab. Invest.* **26,** 47 (1970).
12. J. W. EATON, G. J. BREWER, J. S. SCHULTZ and C. F. SING, in *Red Cell Metabolism and Function* (Ed. G. BREWER), p. 21. Plenum, New York (1970).
13. K. WALTNER, B. TANOS and E. KELEMEN, *Acta med. Hung.* **12,** 147 (1958).
14. R. H. MILLER and S. M. TENNEY, *Proc. Soc. exp. Biol. Med.* **92,** 791 (1956).

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Inhibition of hexose monophosphate shunt by ethanol—An experimental evaluation

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REALIZING the ubiquitous distribution of the enzymes of the hexose monophosphate shunt (HMP) and the importance of this pathway in the probable regulation of the synthesis of nucleotides, Beaconsfield and Reading[1] have attempted to use ethanol as an inhibitor of the HMP. By using ethanol and alcohol dehydrogenase (ADH) in the incubation medium, they have shown that the incorporation of ^{32}P-labeled phosphate into nucleotides of liver slices and mammary tissue was inhibited. The action of the ethanol–ADH system has been interpreted as due to the reduction of NADP and consequent deprivation of this cofactor for the operation of the shunt pathway. This assumption is based on the work of Kini and Cooper,[2] who have shown that NADP, in addition to NAD, is a cofactor for ADH. Selective inhibition of the pentose phosphate pathway by the ethanol–ADH system, if indeed substantiated, is of far reaching significance, since this is in contradiction to the majority of the experimental work which points out that ethanol exerts its inhibitions by reduction of NAD causing a low NAD–NADH ratio.[3,4] All the enzymatic reactions which are favored by a high NAD–NADH ratio are inhibited by ethanol. Paradoxically, Higgins[5] reports a stimulation rather than inhibition of the HMP pathway by ethanol in rat brain minces.

In view of these contradictions in the literature, investigations were undertaken to evaluate the ADH–ethanol system as an inhibitor of HMP using liver slices. Labeled CO_2 derived by catabolism of [1-^{14}C]glucose and [6-^{14}C]glucose in rat liver slices was measured in the presence of ethanol. Also, incorporation of [1-^{14}C]glycine into proteins of liver slices in the presence of ethanol was measured and the magnitude of inhibition by ethanol was compared with the inhibition of catabolism of [1-^{14}C]glucose and [6-^{14}C]glucose.

Wistar male rats (160–180 g) were sacrificed by decapitation, the liver slices were prepared in the cold using a Staddie–Riggs microtome, and incubated at 37° in Krebs–Ringer phosphate buffer containing various concentrations of ethanol, crystalline horse liver dehydrogenase (350 μg/ml) and glucose (5 mM) as [1-^{14}C] or [6-^{14}C]glucose (specific activity, 0·5 μc/3·0 ml of medium). The liver slices (100–120 mg) from 3 to 4 rats were weighed on a torsion balance and transferred into incubation medium. The incubations were carried out in Warburg vessels with KOH in the center wells and O_2 as the gas phase. Labeled CO_2 was collected by absorption into KOH, converted to $BaCO_3$ and assayed by counting on an end window gas flow counter.[6]

Incorporation of [1-^{14}C]glycine into protein was studied in liver slices (500 mg) using Krebs–Ringer bicarbonate medium (pH 7·4). The medium contained 2 mM glycine (417,000 counts/min/3·0 ml of medium), ADH (350 μg/ml) and ethanol (3%). After incubation for 2 hr, the liver slices were collected by precipitation with trichloroacetic acid (15%); a suspension of protein, free of nucleic acids and lipids,[7] was plated on aluminum planchets, counted on an end window gas flow counter and counts were corrected for infinite self-absorption.

Low concentrations of ethanol (3 mM) inhibited $^{14}CO_2$ yields from [1-^{14}C] and [6-^{14}C]glucose by 27 and 67 per cent respectively (Table 1). With 1 M ethanol this inhibition was markedly increased

TABLE 1. EFFECT OF ALCOHOL DEHYDROGENASE AND ETHANOL ON THE CATABOLISM OF LABELED GLUCOSE TO CO_2 IN RAT LIVER SLICES*

Substrate	Ethanol (without ADH) (M)	$^{14}CO_2$ yields ±S. E.	ADH + Ethanol $^{14}CO_2$ yields ±S. E.	P
[1-^{14}C]glucose	nil	46·0 ± 0·85	42·0 ± 1·05	0·01
	0·003	33·8 ± 0·60	34·0 ± 0·95	NS†
	0·005	27·0 ± 0·60	25·0 ± 1·05	NS
	1·000	19·7 ± 1·05	18·0 ± 1·00	NS
[6-^{14}C]glucose	nil	15·0 ± 0·60	18·0 ± 1·05	NS
	0·003	4·9 ± 0·60	5·0 ± 0·50	NS
	0·005	5·0 ± 0·60	4·0 ± 0·40	NS
	1·000	2·4 ± 0·40	2·8 ± 0·15	NS

* Liver slices (100–120 mg) were incubated for 1 hr in 3·0 ml of Krebs–Ringer phosphate buffer (pH 7·4) containing [1-^{14}C]glucose (5 mM) or [6-^{14}C]glucose (5 mM), ADH (350 μg/ml) and ethanol, using O_2 as the gas phase. The yields of $^{14}CO_2$ are expressed as millimicromoles of labeled glucose carbon converted to labeled CO_2 per 100 mg of wet tissue. Values represent averages of five experiments using 3–4 animals in each. Statistical analysis by the Student's *t*-test comparing values obtained with ethanol and ADH + ethanol.

† NS = P > 0·05.

to 80 per cent from [6-^{14}C]glucose, while it was only about 60 per cent from [1-^{14}C]glucose. In none of these experiments did the addition of ADH to ethanol increase the inhibition as reported by Beaconsfield and Reading.[1]

Incorporation of [1-^{14}C]glycine into the proteins of liver slices was inhibited by ethanol at all concentrationsnexamined (Table 2). However, the inhibition was comparable to that of $^{14}CO_2$ formation from [6-^{14}C]glucose at high concentrations of ethanol. Thus the inhibition was about 90 per cent with 1 M ethanol in the medium. Here again the addition of ethanol did not produce any further inhibition of amino acid incorporation.

TABLE 2. EFFECT OF ALCOHOL DEHYDROGENASE AND ETHANOL ON THE INCORPORATION OF [1-^{14}C]GLYCINE INTO RAT LIVER SLICES*

Addition	Ethanol (M)			
	0	0·003	0·060	1·00
nil	73·0 ± 5·7	51·45 ± 6·0	23·5 ± 1·75	4·5 ± 2·5
ADH	60·4 ± 4·9	42·28 ± 2·5	19·33 ± 1·05	5·3 ± 0·29
P values by the Student's *t*-test	>0·05	>0·05	>0·05	>0·05

* Liver slices (500 mg) were incubated for 2 hr in 3·0 ml of Krebs–Ringer bicarbonate buffer (pH 7·4) containing [1-^{14}C]glycine (2 mM, 417,000 counts/min), horse liver dehydrogenase (350 μg/ml) and ethanol, using O_2 and CO_2 (95:5) as the gas phase. Values are expressed as counts per min per milligram of protein ±S.E. of the average of five experiments with 5–6 animals in each.

If ethanol were to reduce NADP selectively, depriving the latter for the operation of HMP, one would expect a greater inhibition of $^{14}CO_2$ production from [1-^{14}C]glucose. The experimental evidence presented here does not indicate such a selective inhibition of HMP. Furthermore, the concentration of ethanol (1 M) employed in the studies by Beaconsfield and Reading[1] was rather high and the observed effects could be of general toxicity. In essential agreement with results presented here (Table 1), Majchrowicz and Quastel[8] have shown that low concentrations of ethanol (3 mM) inhibit

the rate of $^{14}CO_2$ formation nearly 70 per cent from [6-^{14}C] glucose and only 30 per cent from [1-^{14}C]glucose. These results have been interpreted as evidence for the lack of inhibitory action of ethanol on $^{14}CO_2$ derived from labeled glucose via the shunt pathway. The readiness with which NAD rather than NADP is reduced by ethanol in liver has been recently demonstrated.[9,10]

The earliest observation that NADP is an additional cofactor for ADH comes from the work of Pullman *et al.*[11] However, NADP has been shown to have 100 times less affinity than NAD for horse liver ADH, and 30 times less affinity for monkey liver ADH.[2] Furthermore, Dalziel and Dickinson[12] have demonstrated that NAD is firmly bound to ADH and competes with NADP. In view of this kinetic evidence, it is doubtful whether ethanol reduces NADP in preference to NAD and whether the small reduction of NADP is of any consequence in the operation of the hexose monophosphate shunt. A microsomal enzyme system which can reduce NADP in the presence of ethanol is present in the liver, but it requires high concentrations of NADP and ethanol, and is not likely to be of any physiological significance, according to Krebs and Perkins.[10] That the degree of inhibition of incorporation of the labeled amino acid into proteins by ethanol is parallel to the inhibition of $^{14}CO_2$ yields from [6-^{14}C]glucose rather than to that from [1-^{14}C]glucose is further evidence that the major pathways affected are glycolysis and the citric acid cycle, and not HMP. At high concentrations, it appears that ethanol inhibits macromolecular synthesis, probably by interfering with energy metabolism.[8] Therefore, the inhibition of nucleic acid and protein synthesis by ethanol noted by Beaconsfield and Reading[1] is likely due to the general depressant effect of ethanol on cellular mechanisms.

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REFERENCES

1. P. BEACONSFIELD and H. W. READING, *Nature, Lond.* **202,** 464 (1964).
2. M. M. KINI and J. R. COOPER, *Biochem. Pharmac.* **8,** 207 (1961).
3. H. KALANT, *Q. Jl. Stud. Alcohol.* **23,** 52 (1962).
4. T. F. SLATER, B. C. SAWYER and U. D. STRÄULI, *Biochem. J.* **93,** 267 (1964).
5. E. HIGGINS, *Proc. Soc. exp. Biol. Med.* **114,** 591 (1963).
6. E. MAJCHROWICZ and J. H. QUASTEL, *Can. J. Biochem. Physiol.* **39,** 1895. (1961)
7. J. H. QUASTEL and I. J. BIKIS, *Nature, Lond.* **183,** 281 (1959).
8. E. MAJCHROWICZ and J. H. QUASTEL, *Can. J. Biochem. Physiol.* **41,** 793 (1963).
9. J. R. WILLIAMSON, R. SCHOLZ, E. T. BROWNING, R. A. THURMAN and M. H. FUKAMI, *J. biol. Chem.* **244,** 5044 (1969).
10. H. A. KREBS and J. R. PERKINS, *Biochem. J.* **118,** 635 (1970).
11. M. E. PULLMAN, S. P. COLOWICK and N. O. KAPLAN, *J. biol. Chem.* **194,** 593 (1952).
12. K. DALZIEL and F. M. DICKINSON, *Biochem. J.* **95,** 311 (1965).

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Transport of isoniazid across rat small intestine *in vitro*

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MOST drugs are transferred across the mucosa of the gastrointestinal tract as unionized molecules. Rates of transfer are related to the proportion of unionized molecules and their lipid solubility.[1] Using everted sacs of rat small intestine[2] isoniazid transport was investigated *in vitro*. Isoniazid was measured by a semi-specific colorimetric method.[3]