and Aldehyde Metabolizing Systems-IV (Ed. R. G. Thurman), p. 1. Plenum Press, New York (1980).
31. L. A. Videla, H. Ituriaga, D. Bunout, A. Valenzuela, M. E. Pino and G. Ugarte, *Gastroenterology* **82**, 1248 (1982).
32. D. Rollins, A. Larson, B. Steen, K. Krishnaswamy, L. Hagenfeldt, P. Moldeus and A. Rane, *J. Pharmac. exp. Ther.* **217**, 697 (1981).
33. S. Shaw, L. Yayatilleke, W. A. Ross, E. R. Gordon and C. S. Lieber, *J. Lab. clin. Med.* **98**, 417 (1981).

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Ethanol-induced alterations in rat hepatic ammonia metabolism

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Alterations in the structure and physiology of experimental alcoholic liver injury have been the subject of many studies [1–3]. Nevertheless, questions on other toxic effects during ethanol metabolism remain to be answered. The scope for the formation of other toxic agents during ethanol injestion cannot be ruled out and more so, recent reports indicate, without definite conclusions, the interference of ammonia causing neurotoxicity, especially in severe alcoholic liver damage [4] besides alterations in hepatic amino acid and protein metabolism [5]. Hence in the present study we report some of the changes in the metabolic profiles of ammonia as a consequence of repeated loading of high doses of ethanol. This sort of study may help in understanding the chronic damage inflicted on the liver and the possible interplay of synergistic toxic agents like ammonia during ethanol ingestion.

Wistar strain, male albino rats aged 3 months were subjected to a 5-day course of injections i.p. of ethanol (5 g/kg body wt in 10% dilutions). Control animals were treated similarly with physiological saline. The animals were sacrificed by decapitation after the fifth day (24 hr after the last dose of ethanol administration) since withdrawal signs were witnessed from the seventh day. The livers were excised rapidly at a temperature of 0° and homogenates (10% wt/vol.) in required media were prepared, spun at 4000 *g* for 20 min, and the clear cell-free extracts were saved for enzyme assays. Unless specificed, all the steps were carried out at 0–3°. Sucrose homogenates were utilized for assaying lactate dehydrogenase (EC 1.3.99.1) by the method of Nachlas *et al.* [6], asparate aminotransferase (EC 2.6.1.1) as suggested in the Sigma Technical Bulletin [7], glutamate dehydrogenase (EC 1.4.1.2) by the method of Lee and Lardy [8], and glutamine synthetase (EC 6.3.1.2) by the method of Rowe *et al.* [9]. Distilled water homogenate was used for the estimation of AMP deaminase (EC 3.5.4.6) activity by the method of Setlow *et al.* [10] as modified by Wegelin *et al.* [11]. Homogenates prepared in 0.1% cetyltrimethylammonium bromide were used for assaying ornithine transcarbamylase (EC 2.1.3.3), argininosuccinate synthetase (EC 6.3.4.5) by the method of Huggins *et al.* [12], and arginase (EC 3.5.3.1) activity by the method of Beruter *et al.* [13]. To determine the concentration of ammonia, the liver tissue immediately after isolation was immersed in ice-cold 10% trichloroacetic acid, and ammonia concentration was estimated as described by Ward *et al.* [14]. Urea was assayed by the method of Natelson [15] in perchloric acid extracts, while glutamine was estimated by the acid hydrolysis method of Wilcox [16]. The protein concentration in different enzyme sources was analysed following the method of Lowry *et al.* [17] using crystalline bovine serum albumin as the standard. All estimations performed in eight samples were expressed as mean of ± S.D. The statistical analysis of the significance of difference between control and experimental samples was made by the Student's *t*-test. Changes in the basic profiles of animals at the end of the experimental period are shown in Table 1. The body weight and food intake were reduced significantly while the liver gained weight. Recent reports also indicated similar results [18]. These changes associated with declined activities of lactate dehydrogenase and succinate dehydrogenase reflect the reduced energy production and hepatic enlargement. Histological studies showed inflammation of hepatocytes, presence of giant mitochondria, Mallory bodies, bundles of collagen between swollen fatty hepatocytes and depositions of IgA. All these histological features of ethanolic liver have also been reported by earlier workers [19, 20].

Formation of ammonia. Aspartate aminotransferase, NAD-dependent glutamate dehydrogenase and the AMP deaminase were elevated significantly, implying that ammonia formation was stepped up (Table 2). In consonance with the increased activities of these enzymes, ammonia concentration in the ethanolic liver was also increased. Elevated aspartic acid [21] and other dicarboxylic acids [22] in ethanolic liver might have been responsible for transamination of aspartic acid. A similar rise in aspartate aminotransferase induced by ethanol was reported recently [23]. The increased NAD-dependent glu-

Table 1. Basic profiles of animals after the 5-day course of ethanol treatment

Sample	Body weight (g)	Liver weight (g/100g)	Food intake (kcal/day)
Control (8)	193.4 ± 2.7	4.3 ± 0.1	47 ± 0.7
Ethanol-treated (8)	189.2 ± 3.3*	4.8 ± 0.2†	44 ± 0.9†

Values in parentheses show the number of animals taken.
* $P < 0.01$.
† $P < 0.001$.

Table 2. Biochemical responses in liver during repeated ethanol treatment in albino rats

Enzyme/metabolite	Control	Ethanol injected	% change over control
A. ENZYMES			
Lactate dehydrogenase (μmole of formazan)	0.1197 ± 0.004	0.0827 ± 0.006*	−30.90
Succinate dehydrogenase (μmole of formazan)	0.0572 ± 0.001	0.0322 ± 0.0008*	−43.70
Aspartate aminotransferase (μmole of pyruvate)	0.0189 ± 0.001	0.0249 ± 0.002*	31.74
Glutamate dehydrogenase (μmole of formazan)	0.0047 ± 0.0009	0.0072 ± 0.0008*	53.19
AMP deaminase (μmole of ammonia)	0.0022 ± 0.0004	0.0031 ± 0.0003*	40.90
Ornithine transcarbamylase (μmole of citrulline)	0.1881 ± 0.009	0.2412 ± 0.01*	28.22
Argininosuccinate synthetase (μmole of citrulline metabolised)	0.0673 ± 0.01	0.0937 ± 0.004*	39.22
Arginase (μmole of urea)	0.1079 ± 0.0044	0.1921 ± 0.002*	78.03
Glutamine synthetase (μmole of γ-glutamyl hydroxomic acid)	0.0091 ± 0.0003	0.0076 ± 0.0005*	−16.48
B. METABOLITES			
Ammonia	5.5180 ± 1.077	9.5202 ± 0.8402*	72.52
Urea	7.2764 ± 1.5628	11.5321 ± 2.4525*	58.48
Glutamine	8.4036 ± 1.9421	6.3276 ± 1.2525†	−24.70

Enzyme activities are represented in units/mg protein/min. Metabolite levels are expressed in μmole/g wet weight. Values are mean and ± S.D. of 8 samples.

* Student's t-test, $P < 0.001$.

† Student's t-test, $P < 0.01$.

tamate dehydrogenase activity might as well contribute to the well-proven alteration in the NAD:NADH ratio [24]. Though the mechanisms responsible for ethanol-stimulated AMP deaminase have still to be resolved, changes in the metabolism of ATP [25], 5'-nucleotidase [26] and adenylate energy charge [27] may be involved since all these factors are altered in ethanol metabolism and are capable of tuning the AMP deaminase activity [28].

Utilization of ammonia. Excess ammonia will be routed for urea and glutamine synthesis [29, 30] at the expenditure of ATP. The urea cycle enzymes ornithine transcarbamylase, argininosuccinate synthetase and arginase were elevated significantly in ethanolic liver leading to higher levels of urea. The increased utilization of carbamylphosphate and aspartic acid, as reported by Petit and Barral-Alix [21], attests the present results of high urea content and elevated urea cycle enzyme activities. As high ammonia concentration was witnessed despite increased urea formation, it can be suggested that stepped up ureogenesis fails to modify the hepatic ammonia levels. The other pathway involved in ammonia utilization, namely glutamine synthesis, was inhibited and glutamine levels were lowered by 25%. This could have resulted out of increased glutamine utilization by glutaminase [22], which may account for high ammonia concentration in the present study rather than ameliorated glutamine synthesis [31].

In conclusion, the present study indicates hepatic enlargement, structural breakdown of hepatocytes, increased ammonia formation even in the face of stepped up urea synthesis and breakdown in glutamine synthesis. The chances of ammonia manifesting its toxic effects simultaneously with ethanol remain possible because of the prevalence of high ammonia content. An evaluation on the ability of ammonia in potentiating the hepatotoxic effects of ethanol and a further probe into the inter-relationship between ethanol and ammonia will reveal the significance of elevated hepatic ammonia formation during ethanol ingestion.

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REFERENCES

1. E. Baraona, M. A. Leo, S. A. Borowsky and C. S. Lieber, *J. Clin. Invest.* **60**, 546 (1977).
2. Y. Matsuda, E. Baraona, M. Salaspuro and C. S. Lieber, *Lab. Invest.* **41**, 455 (1979).
3. W. J. Jenkins and H. C. Thomas, *Clin. Gastroenterol.* **10**, 307 (1981).
4. S. C. Phillips, *Neuropath. appl. Neurobiol.* **7**, 205 (1981).
5. S. Sharo and C. S. Lieber, in *Biochemistry and Pharmacology of Ethanol* (Eds. E. Majchrowicz and E. P. Noble), p. 383. Plenum Press, New York (1979).
6. M. M. Nachlas, S. P. Marguluns and A. M. Seligmann, *J. biol. Chem.* **235**, 499 (1960).
7. Sigma Technical Bulletin, No. 505 (1979).
8. Y. L. Lee and H. A. Lardy, *J. biol. Chem.* **240**, 1427 (1957).
9. W. B. Rowe, R. A. Ronzio, V. P. Wellner and A. Meister, in *Methods in Enzymology* (Eds. H. Taber and C. B. Taber), Vol. XVII A, p. 900. Academic

Press, New York (1970).
10. B. Setlow, R. Burger and J. M. Lowenstein, *J. biol. Chem.* **241**, 1244 (1966).
11. T. Wegelin, F. A. Manzoli and G. Pane, *Comp. Biochem. Physiol.* **59B**, 55 (1978).
12. A. K. Huggins, G. Skutsch and E. Baldwin, *Comp. Biochem. Physiol.* **28**, 587 (1969).
13. J. Beruter, J. P. Colombo and C. Bachmann, *Biochem. J.* **175**, 449 (1978).
14. J. M. Ward, Jr., A. Roger, A. McNabb and F. M. A. McNabb, *Comp. Biochem. Physiol.* **51A**, 165 (1975).
15. S. Natelson, in *Techniques of Clinical Chemistry*, p. 725. C. C. Thomas, Springfield, Illinois (1971).
16. P. E. Wilcox, in *Methods in Enzymology* (Eds. P. Colowick and N. O. Kaplan), Vol. XI, p. 63. Academic Press, New York (1967).
17. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
18. M. Gascon-Barri, *Metabolism* **31**, 67 (1982).
19. M. A. Swerdlow, L. N. Chowdhory and T. Horn. *Am. J. clin. Pathol.* **77**, 259 (1982).
20. H. Popper, in *Alcohol and the Liver* (Eds. M. M. Fisher and J. Randall), p. 289. Plenum Press, New York (1977).
21. M. A. Petit and I. Barral-Alix, *Biochem. Pharmac.* **28**, 2591 (1979).
22. M. A. Petit, These de Doctorat es-Sciences, Université Pierre et Marie Curie, Paris (1976).
23. S. Sherlock, *Br. med. Bull.* **38**, 67 (1982).
24. T. J. Peters, *Br. med. Bull.* **38**, 17 (1982).
25. M. H. Wisher and W. H. Evans, in *Membrane Alterations as a Basis of Liver Injury* (Eds. H. Popper, L. Bianchi and W. Reutter), p. 127. MTP Press, Lancaster (1977).
26. M. Nishimura and R. Teschke, *Biochem. Pharmac.* **31**, 377 (1982).
27. G. Chapman and D. E. Atkinson, *J. biol. Chem.* **248**, 8309 (1973).
28. J. M. Lowenstein, *Physiol. Rev.* **52**, 381 (1972).
29. S. Sherlock, in *Diseases of the Liver and Biliary System*, 6th edn, p. 334. Blackwell Scientific Publications, Oxford.
30. J. R. Williamson, A. J. Meijer and K. Ohkawa, in *Regulation of Hepatic Metabolism* (Eds. F. Lundquist and N. Tygstrup), p. 457. Academic Press, New York (1974).
31. H. A. Krebs, R. Hems and P. Lund, *Biochem. J.* **134**, 697 (1973).

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Reversal by local anaesthetics of ouabain-induced [^{14}C]ACh and [^{14}C]choline release from synaptosomes

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We have recently shown that ouabain not only causes a significant increase in the release of labelled [^{14}C]ACh from cerebral cortex synaptosomes but also in that of its precursor [^{14}C]choline [1]. It was suggested that this action of ouabain was due to depolarization of the synaptosomal membrane resulting in Ca^{2+}-dependent release of transmitter and, in addition, increased diffusion of the bases down the electrochemical gradient. It was observed that when ouabain was added to synaptosomes in depolarizing (high K^+) media, a further increase in the release of both [^{14}C]ACh and [^{14}C]choline was produced. Ouabain also caused a small, but significant, further increase in the release of [^{14}C]ACh in synaptosomes depolarized by veratrine. These results indicated that ouabain may have an additional effect on the synaptosomal membrane, perhaps a general destabilization or non-specific increase in membrane permeability.

In order to test this possible additional property of ouabain, the effect of two local anaesthetics, procaine and cinchocaine, on the ouabain-induced release of synaptosomal [^{14}C]ACh and [^{14}C]choline was studied. Local anaesthetics are membrane stabilizers so that if ouabain had a destabilizing effect on the synaptosomal membrane, in addition to its inhibition of Na^+–K^+ ATPase then it would be predicted that its action could be partially counteracted by these compounds (to a degree related to their local anaesthetic action). In this paper, we report that two local anaesthetics, procaine and cinchocaine, appear to have this effect.

Materials and methods

Synaptosomes were prepared from scraped guinea-pig cortices by the method of Gray and Whittaker [2]. They were resuspended in 3 mM K^+ medium so that their final concentration was 3–5 mg protein/ml. 3 mM K^+ medium contained 180 mM NaCl, 3 mM KCl, 2 mM $CaCl_2$, 2 mM $MgCl_2$, 8 mM NaPi (pH 7.2) and 10 mM glucose. In the case of 25 mM K^+ medium, the concentration of NaCl was decreased so that the ionic concentration remained unchanged.

Synaptosomes were preincubated with 0.1 μCi/ml (2 μM) [^{14}C]choline for 30 min at 37° during which [^{14}C]ACh was synthesized. They were washed twice with 3 mM K^+ medium containing no Ca^{2+} but 20 μM eserine to remove excess [^{14}C]choline. The synaptosomal pellet was then divided into aliquots of 0.3–0.5 g of brain tissue in Eppendorf microreaction tubes. To each aliquot, appropriate medium (either 3 or 25 mM K^+ medium) was added and where necessary the compounds veratrine (100 μM), ouabain (200 μM), procaine or cinchocaine (both 100 μM). The release was activated by incubating the tubes at 37° for 5 min after which it was terminated by rapid centrifugation in a Janetski TH 11 centrifuge; supernatants and pellets were separated and placed in ice. The supernatants were extracted for ACh and choline by the method of Marchbanks and Israël [3] and Wonnacott and Marchbanks [4]. [^{14}C]ACh and choline were separated by thin layer chromatography and the radioactivity was measured in a Nuclear Enterprises 8312 scintillation spectrometer.