$$CoA + 2\text{-oxoglutarate} + NAD \rightleftharpoons succinyl-CoA + CO_2 + NADH_2$$

$$NADH_2 + MTT \xrightarrow{PMS} NAD + MTT$$

(oxidized form, yellow) (reduced form, blue precipitate)

2-Oxoglutarate, oxoglutarate dehydrogenase, NAD, MTT and PMS, together with acetyl-CoA and choline are layered onto the surface of the electrophoresis plate in a buffered, 1% agar gel (pH 7·0). A blue colouration coincident with ChA activity appears during subsequent incubation at 37° C (30–60 min).

We have used this method for locating ChA activities following separation by isoelectric focusing in Sephadex (G-75) thin layers (Radola, 1969). The results for crude extracts of goldfish skeletal muscle, squid head ganglia, guinea-pig and pigeon brains further demonstrate the heterogeneity of ChA.

REFERENCES

MALTHE-SØRENSSEN, B. & FONNUM, F. (1972). Multiple forms of choline acetyltransferase in several species demonstrated by isoelectric focusing. *Biochem. J.*, **127**, 229–236.

PEARSE, A. G. E. (1957). Intracellular localisation of dehydrogenase systems using monotetrazolium and metal chelation of their formazans. *J. Histochem. Cytochem.*, **5**, 515–527.

RADOLA, B. J. (1969). Thin-layer isoelectric focusing of proteins. *Biochim. Biophys. Acta*, **194**, 335–338.

TUBBS, P. K. & GARLAND, P. B. (1969). Assay of coenzyme A and some acyl derivatives. In *Methods in Enzymology, XIII*, 535–536.

Interaction of alcohol and drugs on psychomotor skills as demonstrated by a driving simulator

M. LINNOILA and M. J. MATTILA

Department of Pharmacology, University of Helsinki, SF-00170 Helsinki 17, Finland

Diazepam may enhance the alcohol induced impairment of human reactive and coordinative skills (Hughes, Forney & Richards, 1965 ; Linnoila & Mattila, 1972) whereas neuroleptics, in low doses, impair attention in particular (Linnoila & Mattila, 1972). The present study was conducted to confirm the validity of our simple laboratory tests (Häkkinen, 1958) as screening procedures for drug-alcohol interactions considered harmful for driving behaviour.

Ninety male drivers, aged 19–21 years, doing their compulsory military service in motorized troops, volunteered for the study. Every subject was carefully tested before entering the army. They were distributed in nine test groups 10 subjects in each. The drugs, given double blind in identical capsules, were: 10 mg of diazepam, 25 mg of codeine phosphate, or 750 mg of isoniazid. Drugs were administered in combination with placebo or similar alcoholic bitter drink, the amount of ethanol being 0·5 g/kg. The subjects were asked to assess their capacity of performance and the quality of their drug and drink.

The main simulator device was a Sim-L-Car with one point system shadow projection. Clutch, brake, gears, flashing lights and changes in steering and speed were recorded in a number of individual movements. The device also permitted recording of the lateral distance of the vehicle to objects in the path of the car. An eight-channel recorder registered continuously certain characteristics. Two sets of counters, 15 each, could be switched on in varying intervals. Pulse frequency and reaction time were also measured. The subjects and the screen were TV-filmed. The process of testing was programmed by punched cards. Emergency situations were simulated by a car suddenly entering from a side road. The driving period was 40 min starting 30 min after the drug intake.

Alcohol alone increased the collision frequency and made the subjects prone to ignore instructions and traffic rules. Diazepam alone increased the collision frequency and so did codeine. Diazepam given in combination with alcohol resulted in a further increase

in the number of collisions and negligence of the rules, but a new phenomenon was serious steering errors. Codeine potentiated alcohol in the same way as diazepam, and both codeine and diazepam reduced the tachycardia induced by an emergency situation. Isoniazid alone exerted minimal effects and only slightly enhanced alcohol effects. Generally, the most sensitive variables to the drug effects were changes in the steering direction, flashing lights, brakes, and clutching.

The results confirm our previous results concerning the harmful effects of combining diazepam and alcohol on driving skill. They also suggest that relatively simple laboratory procedures could be used to predict drug interactions which reduce driving skill.

REFERENCES

HÄKKINEN, S. (1958). Traffic accidents and driver characteristics. A statistical and psychological study. *Teknillinen Korkeakoulu, Helsinki.*

HUGHES, F. W., FORNEY, R. B. & RICHARDS, A. B. (1965). Comparative effect in human subjects of chlordiazepoxide, diazepam and placebo on mental and physical performance. *Clin. Pharmacol. Ther.*, **6**, 139–145.

LINNOILA, M. & MATTILA, M. J. (1972). Problems of drugs and driving: interaction of alcohol and psychotropic drugs. *Invited communication, Clinical Pharmacology Symposium, 5th Int. Congr. Pharmac., San Francisco.*

Lignocaine metabolism in man

K. K. ADJEPON-YAMOAH and L. F. PRESCOTT

University Department of Therapeutics, The Royal Infirmary, Edinburgh EH3 9YW

Metabolites of lignocaine with potential antiarrhythmic activity include ethylglycylxylidide (EGX) and glycylxylidide (GX). Because of methodological difficulties, it has not been possible previously to measure their concentrations in the plasma of patients treated with lignocaine and there is little data on their urinary excretion pattern. A specific and sensitive gas-liquid chromatographic method has been developed for the simultaneous estimation of the three compounds in human plasma and urine.

Lignocaine metabolism was studied in four fasting healthy volunteers given lignocaine hydrochloride 400 mg orally and 200 mg intramuscularly on separate occasions. Venous blood and urine samples were collected at regular intervals. Urine pH was not controlled. Preliminary studies have also been carried out in hospital patients.

Following oral administration, mean peak plasma concentrations (μg/ml, mean $\pm$ S.E.) were $0{\cdot}81 \pm 0{\cdot}02$ for lignocaine, $0{\cdot}6 \pm 0{\cdot}11$ for EGX and $0{\cdot}26 \pm 0{\cdot}05$ for GX. Maximum concentrations were reached at 0·5 h, 1 h and 2 h respectively. The apparent mean plasma half life was 1·4 h for lignocaine, 2·8 h for EGX and about 15 h for GX.

Following intramuscular injection, mean peak plasma concentrations were $1{\cdot}25 \pm 0{\cdot}09$ for lignocaine at 0·5 h, $0{\cdot}18 \pm 0{\cdot}02$ for EGX at 2 h and $0{\cdot}12 \pm 0{\cdot}02$ for GX at 6 hours. The apparent mean plasma half-life was 1·7 h for lignocaine and 4·6 h for EGX. The plasma half-life of GX could not be determined since the mean peak concentration occurred at 6 h and blood samples were taken for only 8 hours.

Lignocaine and EGX could not be detected in the urine after 24 h and 36 h respectively (limit of detection 0·02 μg/ml). In contrast, GX was still easily measurable after 48 h and the maximum urinary excretion occurred in the period 10–24 h after intramuscular injection. Cumulative urinary excretion of these compounds is shown in Fig. 1. The observation of Boyes & Keenaghan (1972), that 4-hydroxyxylidine is a major urinary metabolite of lignocaine has been confirmed.

These studies indicate that GX is an important metabolite of lignocaine and has a very long biological half-life. Cumulation of GX may be relevant to the therapeutic and toxic effects of lignocaine. As much as 19% of a dose of lignocaine has been recovered as GX in the urine of a patient in 24 hours.