The experimental procedure was as follows. Dorsal roots L6 to S2 were sectioned under halothane anaesthesia in 6 animals. In a further 4 animals (mock operated), the cord was exposed but the roots left intact. After a recovery period of 6 days, appropriate electromyograms were recorded to ensure that dorsal root pathways were functional in control animals but not in DRS animals, and that no gross cord damage had resulted from the surgical procedures. The animals were then killed by cervical fracture and the spinal cord vasculature perfused with ice-cold saline via the thoracic aorta. The deafferented cord was then removed together with an additional section from L2–L5. These were extracted separately in trichloracetic acid (10%) and analysed on a Technicon automatic amino acid analyser. Control experiments were performed to determine the reproducibility of the extraction and analysis procedures and histological studies confirmed degeneration after DRS.

Amongst the 17 amino acids studied, eleven were found to have a significantly higher concentration in the lower, as compared with the upper, section of the cord of mock operated control animals. Such a distribution is compatible with the greater proportion of grey matter in the lower part of the cord where substances with a neurotransmitter role would have a relatively higher concentration. However, only two amino acids, aspartate and cystathionine, were significantly ($P<0.05$) reduced (48% and 51% respectively) by DRS. Although glutamate and GABA levels were substantially reduced (26% and 36% respectively), these changes were not significant. Glycine was little affected (+4·9%). In interpreting these results it should be noted that small changes in the concentration of a given neurotransmitter amino acid following DRS may be masked by high endogenous levels of that substance subserving some other purposes.

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A method for the induction of dependence to ethanol in mice

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If ethanol dependence is to be shown to obey normal pharmacological criteria of tolerance and dependence, then an experimental model must be available. For many years investigators have concentrated upon developing techniques for inducing ethanol preference in laboratory animals, although withdrawal signs do not appear consistently on cessation of drinking (Myers & Veale, 1971).

We have now developed a model based on the inhalation of ethanol vapour alone which consistently produces ethanol tolerance and dependence in mice.

Groups of thirty male mice (18–22 g) were exposed to increasing concentrations of ethanol in the inspired air for 7–10 days. The ethanol concentration in inspired air was measured by gas liquid chromatography using polyethylene glycol 20 m 20% with chromosorb 101 80–100 on a 9 ft glass column, and was increased from 15–25 mg/l. initially to a final level of 40–60 mg/l. after ten days. Ambient temperature was maintained at 27° C±1° C to prevent hypothermia. At intervals throughout the induction of dependence, blood and brain ethanol and acetaldehyde levels were measured (Duritz & Truitt, 1964). Brain ethanol levels were found to be closely related to blood levels, which rose gradually throughout the experiment. A representative group of thirty mice had 150±30 mg of ethanol/100 ml of blood on day 2 to 400 mg±40 mg ethanol/100 ml blood on day 10.

During the inhalation of ethanol, treated mice showed locomotor depression and ataxia. They also ate and drank less than control mice. These differences were not large, and treated mice showed little change in weight compared to controls. The death rate of treated mice was between 10 and 15%.

It was necessary to increase the concentration of ethanol in the inspired air throughout the experiment in order to maintain the behavioural depression and ataxia. The concentration of ethanol in the cage on the tenth day was lethal to naive mice within 8 hours.

The final blood levels of ethanol obtained in mice treated for ten days caused coma and eventually death in naive mice.

Ethanol administration for 10 days markedly increased the rate of elimination of ethanol from blood and also from the brain.

These observations are thought to provide evidence for both metabolic and pharmacological tolerance during the administration of ethanol in this way.

Evidence of dependence on ethanol was sought by close observation of the behaviour of mice after the ethanol concentration in the inspired air was brought down rapidly to near zero levels. This could be accomplished in approximately ten minutes. Within 15 min the mice began a period of often intense locomotor excitation. This reached a peak after about 75 min and declined thereafter, to be followed by a period of locomotor depression. Two hours after ethanol withdrawal, treated mice showed fine tremor and piloerection. A characteristic convulsion (Goldstein & Pal, 1971) could be elicited by holding a withdrawn mouse by the tail. These signs of withdrawal usually persisted until some 10 or 12 h after ethanol withdrawal. Objective evidence of some of these changes has been obtained using an Animex Activity Meter type S.

Goldstein & Pal described similar changes, but with a more protracted time course.

It is concluded that the administration of ethanol in this way fulfils the conditions for a valid model of ethanol dependence.

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A stain for the detection of choline acetyltransferase

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Evidence for the existence of choline acetyltransferase (EC 2.3.1.6.) (ChA) in multiple forms has been obtained by column isoelectric focusing (Malthe-Sørenssen & Fonnum, 1972). While the physiological significance of this observation remains to be clarified, the value of the method for characterizing ChA in different tissues is evident. We describe here a stain for ChA which, together with thin layer separative methods, allows rapid characterization of ChA from both central and peripheral tissues.

CoA produced in the reaction of ChA with acetyl-CoA and choline is localized, as it is formed, by reaction with oxoglutarate dehydrogenase (EC 1.2.4.2.) (Tubbs & Garland, 1969) in the presence of thiazolyl blue (3,(4,5-dimethylthiozalyl-2)-2)-2,5-diphenyl monotetrazolium bromide; MTT) and phenazine methosulphate (N-methylphenazonium methosulphate; PMS) (Pearse, 1957).