medical and nursing staff (both medical and psychiatric) at a district general hospital, without a specialist alcohol treatment unit, completed, anonymously, questionnaires evaluating their attitudes to treatment (Caine et al. 1989, The Claybury Selection Battery Manual, NPSA-Nelson, Windsor, UK). Questionnaires from patients referred with alcoholism, from medical and psychiatric sources, were similarly scored. In addition, patients completed a treatment expectancy questionnaire. Both groups of staff scored similar values, on a psychological-organic scale. The mean ± SE scores for the staff (medical 53.7 ± 1.0, nursing 54.7 ± 0.8) were significantly less than those of the patients (60.0 ± 2.6, p < 0.001, p < 0.05) indicating that the latter have a more organic than psychological perception of their condition. Moreover, patients referred from psychiatric sources have significantly higher scores (74.2 ± 3.7) than those referred from medical services (65.5 ± 2.6). Statistical analysis of the patients' results on the two questionnaires revealed a high correlation between them (r = 0.84, p < 0.001). It is suggested that the patient-staff discrepancy of attitudes to treatment may have important therapeutic implications. Attitudes and treatment expectancies such as those exhibited by alcohol dependent patients may contribute to the low compliance rate in treatment centres where medical intervention is absent.

62 ANALYSIS OF ORGANIC ACIDURIAS BY PROTON - "SPIN-ECHO NUCLEAR MAGNETIC RESONANCE ('H-NMR)"
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Recently 'H spin-echo NMR spectra have been obtained from the urine of patients with various inborn errors of metabolism, eg, maple-syrup urine disease (Iles et al, Biochem Soc Trans, in press), glutaric-aciduria, proponionic-acidaemia and methyl-malonic aciduria. Using this technique characteristic spectra of proton containing organic metabolites at a concentration of 0.5 M were produced within 5 min.

The samples (0.5 ml) of urine were placed in a 5 mm NMR tube and run in a Brucker WH 400 MHz NMR spectrometer in the Chemistry Department, Queen Mary College, at room temperature. A 90° - 2° - 180° - t (spin-echo pulse sequence as used then (Brown et al, 1977, FEBS letters 82, 12-16); typically 240-360 pulses were required (4-6 min) to give interpretable spectra. Peaks identified in normal urine included creatinine, citrate, hippuric acid and various amino acids. In the three organic acidurias studied characteristic spectra were obtained which may be used as a 'fingerprint'. The advantages of this method are firstly that no sample preparation (eg, separation or derivatisation) is required and secondly in the diseases studied it is rapid (given access to a suitable spectrometer).

Finally, a potential screening of all organic 'H-containing metabolites in the millimolar concentration range is given including acids, bases and neutral compounds. Thus it may be applied to unexplained metabolic acidoses, disorders in purine metabolism and drug metabolism.

63 NEUROPEPTIDE Y IN MAMMALIAN CARDIAC TISSUE: EFFECTS ON ISOLATED PERFUSED RABBIT HEART
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Neuropeptide Y (NPY) a recently isolated peptide from porcine brain, has been demonstrated within neurones outside the central nervous system and appears to be associated with sympathetic nerves. High concentrations of this peptide have been measured in extracts of heart tissue from various species including rat, dog and mouse, using a recently developed specific radioimmunoassay. Concentrations throughout the regions of the heart were relatively uniform (being 34.1 ± 0.4 pmol/g in the left ventricle) except in the region of the AV node and coronary vessels, where levels were significantly higher. Isolated perfused rabbit hearts (Langendorff technique) were used to investigate the possible direct physiological role of this peptide in controlling heart rate, force of heart contraction and myocardial perfusion. Addition of NPY at a dose of 100 pmol to the perfusate resulted in a prompt reduction in myocardial perfusion to 57.5 ± 15.2% of basal over the first minute. This reduction in myocardial perfusion was maintained over the following five minutes and recovery was not complete up to fifteen minutes after the dose. A dose-dependent delayed negative inotropic effect was consistently demonstrated which was characterised by slow onset and recovery, and may be secondary to reduction in perfusion. The presence of high concentrations of this potent peptide, therefore, in all regions of the heart tends to suggest that it may have an important regulatory role in cardiac physiology and pathophysiology.

64 ANTIGEN BRONCHIAL PROVOCATION CAUSES AN INCREASE IN PLASMA ADENOSINE LEVELS IN ASTHMA.
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Adenosine (A) is a purine nucleoside formed from AMP. As an extracellular mediator, it stimulates specific cell surface receptors to increase (A) or decrease (A) cellular levels of cyclic AMP. A and AMP
are potent bronchoconstrictors in asthmatic but not normal subjects. Antigen (ag) challenge and hypoxia stimulate release of adenosine from animal lung tissue in vitro. Since these conditions are pertinent to the pathophysiology of asthma, we have measured plasma levels of A and its metabolite inosine (I) following ag challenge in 7 allergic asthmatic subjects. On two occasions subjects were challenged with an inhaled aerosol of house dust mite ag. At 3 time points before, and at 2, 5, 10, 15, 30, 45 and 60 min after challenge measurements of FEV1 were made and blood was taken from an indwelling venous cannula for measurement of plasma A and I levels. Plasma was separated at 4°C, deproteinised with perchloric acid, neutralised and the cis-diols isolated by phenylboronate chromatography. A and I eluted from the affinity gel were separated by HPLC and quantified by UV absorbance at 254 nm. (Gehrke C.W. et al, J. Chromatog. 150,455-476: 1978). Nucleoside recovery from plasma was over 90%. Following antigen challenge plasma adenosine increased from a baseline of 5.0±0.8 ng/ml (mean ± SEM) to 7.9±1.3 ng/ml at 2 min (p<0.01) and reached a maximum of 15.0±2.8 ng/ml (p<0.01) at 45 min. No significant change in plasma inosine occurred. Antigen challenge caused a progressive fall in FEV1, reaching 25% at 15 minutes. These data suggest that adenosine may be a newly recognised endogenous mediator of antigen-induced bronchoconstriction in asthma.

65 DIET AND NORADRENALINE INDUCED THERMOGENESIS IN MASSIVE OBESITY

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We have investigated diet-induced and noradrenaline (NA) induced thermogenesis in massive human obesity. Twenty-one obese subjects (mean Obesity Index 45.1) and 5 lean control subjects (mean Obesity Index 20.1) were studied. Metabolic rate was measured by the ventilated hood technique and results expressed as energy expenditure in kcals/24 hours. Measurements were made under resting conditions and in response to a 45 minute infusion of NA (0.1 µg/kg ideal body weight) and in response to a 45 minute infusion of NA (0.1 µg/kg ideal body weight). Metabolic rate was significantly higher in the obese (1195 kcals/24 hours; P<0.0006, Mann Whitney Test). The NA induced rise in metabolic rate was higher in the obese whether analysed by peak rise, area under curve of rise, or individual time points.

66 ACETALDEHYDE IS NOT DIRECTLY HEPATOTOXIC IN ALCOHOLIC LIVER DISEASE

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There is increasing evidence that immunological mechanisms may play a role in the pathogenesis of alcoholic liver damage. If such mechanisms are involved, then the effects of alcohol abuse should be manifest on the surface of the hepatocyte membrane in order to initiate the immune response. Acetaldehyde, the immediate metabolite of ethanol, is found in high levels in the serum during ethanol abuse and may be the hepatotoxic factor. We have therefore studied the effects of acetaldehyde on isolated rat hepatocytes with particular reference to the hepatocyte plasma membrane.

Methods

Hepatocyte plasma membrane vesicles were prepared on discontinuous sucrose gradients and purity assessed by assay of enzyme markers. The binding of acetaldehyde to the membranes via intermediary Schiff base formation was studied by reductive methylation using 3H labelled sodium cyanoborohydride. The effects of acetaldehyde on (a) plasma membrane (b) hepatocyte function was studied by measuring (a) LDH leakage and alanine transport (b) urea synthesis and gluconeogenesis in isolated hepatocytes incubated with acetaldehyde.

Results

The binding constant of acetaldehyde to hepatocyte plasma membranes was 13.2 ± 4.1 µM (SEM) and 38 ± 7.7 nmols were bound/mg membrane protein. Plasma membrane function was not influenced by acetaldehyde concentrations up to 20 µM. Gluconeogenesis and urea synthesis were not influenced until acetaldehyde concentration exceeded 10 µM.

Conclusions

Acetaldehyde binds to hepatocyte plasma membranes. The binding does not influence membrane function or cell metabolism but may influence membrane structure. The results are compatible with the hypothesis that any injurious effect of acetaldehyde may be mediated by the immune system and is unlikely to be a direct toxic effect on cellular metabolism.