TRANSPORT of 250 ± 20 μmol/l). Sodium transport has been studied in normal human leucocytes, in vitro, during manipulation of the external zinc concentration from 0-75 μmol/l to 90 μmol/l. There was a progressive increase in the rate constant for total sodium efflux from 3-03 ± 0-14 at 0-75 μmol/l to 4-11 ± 0-13 at 90 μmol/l. Intracellular sodium content did not change significantly. Sodium influx rises in a similar manner with increasing external zinc concentrations (181 ± 17 μmol of Na kg⁻¹ dry weight at [Zn] 0-75 μmol/l to 1250 ± 20-9 at [Zn] 90 μmol/l).

The external zinc concentration needs to be controlled in studies of sodium transport in human leucocytes.

9. THE EFFECT OF ZINC ON LEUCOCYTE SODIUM TRANSPORT

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Sodium transport has been studied in normal human leucocytes, in vitro, during manipulation of the external zinc concentration from 0-75 μmol/l to 90 μmol/l. There was a progressive increase in the rate constant for total sodium efflux from 3-03 ± 0-14 at 0-75 μmol/l to 4-11 ± 0-13 at 90 μmol/l. Intracellular sodium content did not change significantly. Sodium influx rises in a similar manner with increasing external zinc concentrations (181 ± 17 μmol of Na kg⁻¹ dry weight at [Zn] 0-75 μmol/l to 1250 ± 20-9 at [Zn] 90 μmol/l).

The external zinc concentration needs to be controlled in studies of sodium transport in human leucocytes.

10. THE EFFECTS OF MEAL FREQUENCY ON BODY COMPOSITION AND HUNGER DURING WEIGHT REDUCTION

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It has been suggested (Fabry, 1976, In: Energy Balance in Man, pp. 297–303. Ed. Apfelbaum, M.) that isoenzymic diets fed in large meals (gorging) promote greater energy retention than frequent small meals (nibbling), and hence the nibbling mode should be more effective in reducing diets. Nineteen overweight volunteers studied in a metabolic ward for 3 weeks on a protocol approved by the hospital ethical committee. The energy value of the diet averaged 3·22 ± 0·21 MJ daily throughout. All patients were fed a similar diet with three meals per day in the first week (I). During the second or third week, isoenzymic food was arranged either as gorging (G) with 75% energy in a single mid-day meal, or nibbling (N) with five meals of 13·25% total energy each. Nine patients were fed N–G, and 10 were fed G–N in the last two weeks.

There were significant decreases with time in the rate of weight loss (week 1 vs week 3, P < 0·01) and in fasting resting metabolic rate measured by indirect calorimetry (week 1 vs weeks 2 and 3, P < 0·001) but there was no significant difference between the week N vs the week G.

Negative nitrogen balance was highest in week I and decreased significantly in the subsequent weeks; week I vs week 2 (P < 0·001); week I vs week 3 (P < 0·01). Week I vs week N showed a highly significant decrease in negative nitrogen balance (P < 0·001) which was less marked in week I vs week G (P < 0·02). Week N vs week G showed a significantly less negative nitrogen balance (P < 0·01).

Subjective ratings of hunger and desire to eat decreased significantly between week I and week 3 (P < 0·02) but were significantly lower in week N vs week I (P < 0·01) but not significantly different in week G vs week I.

During a reducing diet the gorging mode was associated with greater nitrogen loss and greater hunger than the nibbling mode, but there was no significant effect on weight loss or metabolic rate.

11. THE DETECTION OF CYSTEINE-HOMOCYSTEINE MIXED DISULPHIDE IN FASTING PLASMA OF NORMAL MAN AND THE DIFFERENCE IN LEVELS FOUND BETWEEN MEN AND WOMEN

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Abnormalities of methionine metabolism are responsible for homocystinuria in which arteriosclerotic disease in infancy and childhood is a prominent feature. In this condition cysteine–homocysteine mixed disulphide is detected routinely in fasting plasma before treatment. It has not been identified in the fasting plasma of normal man.

Using a highly cross-linked resin with a JEOL Amino Acid Analyser, we measured plasma amino acids in blood obtained after an overnight fast in 39 normal subjects. There were 20 men and 19 women; ages were from 23 to 50 years. All the women were pre-menopausal.

Small amounts of cysteine–homocysteine mixed disulphide were detected in the fasting plasma of all 39 normal subjects. The mean fasting concentration was 3·2 ± 0·4 μmol/l (±SD = 0·8) and varied with a range from 1·6 to 4·8 μmol/l. The other neutral and acidic amino acids were within the accepted normal range. The mean value for men (±SD) of 3·7 ± 0·4 μmol/l was significantly higher than that for women, which was 2·6 ± 0·6 (P < 0·001). There was no difference in age.

The study shows that in the normal metabolism of methionine all of the homocysteine formed does not react immediately with serine to produce cystathionine, or become remethylated to methionine; some must combine with cysteine to form measurable amounts of mixed disulphide. The study also shows that under the age of 50 years fasting mixed disulphide levels are higher in men than in women. Since prolonged homocysteine infusions produce endothelial damage and atherosclerosis in baboons, the present findings could be relevant to an understanding of the pathogenesis of vascular disease.

12. THE ROLE OF GLUCOCORTICOIDS AND GLUCAGON IN THE METABOLIC RESPONSE TO MODERATE INJURY

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The relative importance of insulin, glucocorticoids and glucagon in mediating the catabolic response to injury remains controversial. Circulating concentrations of several hormones and metabolites were therefore measured in six patients receiving saline infusions after laparotomy and compared with findings in five normal volunteers fasting for a similar period. After operation blood glucose concentration and nitrogen excretion were significantly higher and blood alanine and ketone body metabolite concentration in each group. In control subjects operation blood glucose concentration and nitrogen excretion was 0·45, P < 0·05. Glucagon correlated with glycerol (r = 0·84), while cortisol correlated with ketone bodies (r = 0·46).

Distinct changes were observed after operation. Insulin then correlated with glucose (r = 0·72), pyruvate, (r = 0·46) and alanine (r = 0·40) and negatively with glucagon (r = 0·37), ketone bodies (r = 0·59) and triglyceride (r = 0·54).
Glucagon correlated with glucose (r = 0.66), lactate (r = 0.36), pyruvate (r = 0.4), and insulin (r = 0.57) but negatively with ketone bodies (r = -0.46) and triglycerides (r = -0.62). Cortisol correlated with glycerol (r = 0.37). Urine nitrogen excretion correlated with glucagon after operation (r = 0.72) more strongly than in controls (r = 0.52).

These findings show increased nitrogen excretion after moderate injury, associated with increased gluconeogenesis from amino acids. After injury, hormones appear to influence metabolism more strongly, while in fasting, relationships between metabolites appear important. After injury, even at low levels insulin inhibits lipid mobilization. Glucagon probably mainly affects the liver stimulating gluconeogenesis and ureagenesis. Cortisol appears to play a lesser role in moderate injury than we noted after severe injury, where it appeared to mediate the mobilization of peripheral protein and lipid stores (Batstone, Alberti, Hinks, Smythe, Laing, Ward, Ely & Bloom, 1976, Burns, 2, 204).

13. SUCCINIMIDE IN THE TREATMENT OF PRIMARY HYPEROXALURIA

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There is no universally satisfactory therapeutic agent for the control of urinary oxalate excretion in primary hyperoxaluria, although pharmacological doses of pyridoxine reduce the urinary oxalate excretion in some patients. Succinimide has been claimed to cause a significant reduction in urinary oxalate levels in patients with oxalate nephrolithiasis not due to primary hyperoxaluria (Thomas et al., 1971, Annales de Urologie, 5 U-145). We have carried out a trial of succinimide in three stone-forming children with type 1 primary hyperoxaluria who are not responsive to pyridoxine therapy.

Baseline urinary oxalate, glycollate and other organic acid levels were determined over 10 days during which no medication was given but a high urinary flow rate maintained. The patients were then given 160 mg/kg body weight succinimide orally per day. Urine was collected for a further 10 days before the patients were discharged on the same dose. Oxalic acid was determined by isotope dilution analysis and by gas/liquid chromatography, which also enabled glycollic and other organic acids to be measured.

During the first 10 days of the trial, there were no significant changes in oxalate or glycollate excretion. The following levels (mean mol/24 h anhydrous oxalic acid, with SD) were found:

**Patient 1 Pre-treatment:** 2.44, 0.54
**Succinimide:** 2.51, 0.77

**Patient 2 Pre-treatment:** 5.69, 0.59
**Succinimide:** 1.79, 0.21

**Patient 3 Pre-treatment:** 1.71, 0.30
**Succinimide:** 1.79, 0.24

The results of a longer term follow-up over 10 weeks on this regimen will be presented.

It appears that the proposed mechanism of Thomas et al. whereby succinimide acting, through succinate, as a substrate in the TCA cycle is able to stimulate the complete oxidation of glyoxylic acid to CO₂ rather than to oxalic acid is not significant in these patients. This investigation was approved by the Northwick Park Hospital Ethical Committee.

14. ORGANIC ACIDS IN HUMAN AMNIOTIC FLUID AND THE PRENATAL DIAGNOSIS OF THE ORGANIC ACIDURIAS

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Prenatal diagnosis of inborn errors of metabolism by direct chemical analysis of amniotic fluid for accumulating metabolites derived from the foetus offers the great advantage of rapidity over the more conventional determination of enzyme activities in cultured cells. Reliable diagnoses depend on comprehensive but specific methods and a detailed knowledge of the patterns and levels of metabolites normally present in the fluid.

We have examined amniotic fluid, obtained at 15-20 weeks gestation from 40 uncomplicated pregnancies with a normal outcome, for organic acids using quantitative and semi-quantitative extraction techniques, gas chromatography, and mass spectrometry. The major organic acids observed (μg/ml of fluid, mean ± SD) were: lactate (870 ± 122), pyruvic (7.6 ± 2.8), 2-hydroxybutyric (18.5 ± 5.7), 3-hydroxybutyric (9.9 ± 4.3), succinic (1.2 ± 0.4), fumaric + glyceric (3.1 ± 1.6), malic (3.1 ± 0.9), pyrogulamic (17.8 ± 7.2), erthyritic + threonic (1.9 ± 0.9), 2-oxoglutaric + 3-hydroxy-3-methylglutaric (2.8 ± 3.1), citric (74.4 ± 15.6), 4-hydroxyphenyl-lactic (0.9 ± 0.6) and palmitic (1.6 ± 0.3). Other acids observed included 3-hydroxyisovaleric (0.4 ± 0.2), acaetoic (0.5 ± 0.3), 2-oxoacaproic (0.4 ± 0.2), 3-deoxyretroen (0.2 ± 0.1) and stearic (0.1 ± 0.1).

These results provide an aid to the prenatal diagnosis of the organic acidurias. The methods used permit the detection and quantification of all acidic metabolites potentially present in the fluid and will allow the diagnosis of known diseases for which the pregnancy is at risk and abnormal metabolites accumulate.

In such cases, where the nature of the accumulating metabolite(s) is known (for example in methylmalonic aciduria), the diagnosis is made more specific by use of selected ion monitoring with the mass spectrometer for characteristic ions. This approach should also permit the prenatal diagnosis of other organic acidurias that are expressed in the newborn infant at birth and allows the exploratory analysis of amniotic fluid for possible foetal inborn errors of metabolism in mothers with a history of unexplained early neonatal deaths or miscarriages. Wherever possible, confirmatory enzyme assays on cultured cells are advised, and mass spectrometric identification of accumulated metabolites is considered essential.

15. CORRECTION OF RENAL HYPERTENSION IN THE RAT BY LONG-TERM INFUSION OF ANGIOTENSIN INHIBITORS

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As in earlier studies in the rat intravenous injection of saralasin (180 μg/kg in this study) or converting enzyme inhibitor (1 mg/kg) produced a variable and usually small reduction of blood pressure in conscious rats with two-kidney hypertension of mean duration 42 days. However, prolonged infusion of saralasin (at 10 μg min⁻¹ kg⁻¹) or converting enzyme inhibitor (6.9 μg min⁻¹ kg⁻¹) gradually reduced blood pressure to normal in 15 of 16 rats. Control infusion of saralasin in normal animals or of glucose in normal and hypertensive animals did not reduce blood pressure. Plasma renin concentration correlated significantly with the early but not with the later fall of blood pressure (r = 0.64, P < 0.02 for the first). Plasma concentrations of renin and angiotensin II were closely related (r = 0.91, P < 0.001) except in rats receiving converting enzyme inhibitor, when angiotensin II was relatively reduced. The reduction of pressure was not associated with increased urinary sodium excretion.

16. EFFECT ON CONVERTING ENZYME INHIBITOR AND SARALASIN OF THE REFLEX CONTROL OF BLOOD PRESSURE

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