renal transport defect is easily proved by determination of the clearance of the four affected amino acids. These are all grossly increased up to values sometimes equal to the glomerular filtration rate despite plasma concentrations of the amino acids which are either normal or low. Proof of defective intestinal absorption has been given by: (a) failure of concentration of the amino acids when intestinal mucosa from cystinurics is incubated *in vitro*; (b) lower plasma concentrations of the affected amino acids after their oral ingestion, combined with a normal plasma decay curve after intravenous injection; (c) detection of unabsorbed amino acids in faeces, or of products of their bacterial degradation both in faeces and in urine; (d) intubation experiments with direct demonstration of an abnormally slow disappearance of the affected amino acids from the gut lumen. Bacterial degradation products from lysine include pentamethylenediamine (cadaverine) and piperidine, and from arginine, citrulline, ornithine, tetramethylenediamine (putrescine) and pyrrolidine. All these compounds have been detected in excess in the urine, the faeces, or in both, of cystinuric patients. Dietary cystine is mainly degraded to inorganic sulphate. Urinary sulphate increases later after oral administration of cystine in cystinuric patients as compared with controls, owing to the delay in the unabsorbed amino acid reaching the colon.

Cystinuria has been shown to be due to at least three abnormal allelic genes at the same chromosomal locus. The easiest method of differentiation between different types of cystinuria is by examination of the urine of heterozygotes in the affected families. In the completely recessive form of the disease, the urine of heterozygotes is normal, whereas in the incompletely recessive type, hetero-

zygotes excrete excess of cystine and lysine, but not of arginine or ornithine. The cystine output is too low for crystallization and stone formation to occur. In the former, more common type the intestinal transport defect of homozygotes is especially severe. Despite the fact that lysine is an essential amino acid, cystinuric patients show no obvious nutritional impairment. This apparent paradox has now been explained by the dual mechanisms of absorption by the gut of the products of protein digestion. Free amino acids are absorbed by an active transport process, with concentration of the amino acid within the mucosal cell above that either in the lumen or in the portal capillary blood. In addition, oligopeptides may be taken up by the cell by a process independent of that involving the free amino acids. The peptides are then hydrolysed either in the brush border or in the cell interior, and delivered to the portal capillary blood as the constituent free amino acids. Only the free-amino-acid transport mechanism is impaired in cystinuria, allowing normal nutrition to be maintained by a normal capacity for oligopeptide uptake. Thus, after ingestion of the dipeptide lysylglycine, plasma lysine in cystinurics increases more rapidly than after oral intake of a corresponding equimolecular mixture of free lysine and glycine. Similarly, plasma concentrations of both arginine and of lysine increase more rapidly after oral casein than after a mixture of free amino acids derived from the protein by complete hydrolysis.

In summary, although cystinuria is a somewhat rare disease causing only a small proportion of urinary calculi, it is of disproportionate interest and importance in the study of the mechanisms of amino acid transport, a process that is still poorly understood.

COMMUNICATIONS

Influence of Plasma Lipids on the Activity of Lipoprotein Lipase

By PHILIP E. BRUMBY. (*National Institute for Research in Dairying, Shinfield, Reading, Berks., U.K.*)

Bovine milk lipoprotein lipase hydrolyses the triglycerides of human plasma lipoproteins but not those of bovine or guinea-pig plasma lipoproteins. Resistance to hydrolysis may not be entirely due to the protein component, though this is known to be important in relation to the activity of lipoprotein lipases (Fielding, 1970b; Fielding, Lim & Scanu, 1970; LaRosa, Levy, Herbert, Lux & Fredrickson, 1970) as an activated emulsion of the lipid from bovine plasma lipoprotein is also a poor substrate. Further, incorporation of bovine plasma lipid at a

concentration of 30% into a soybean-oil emulsion (89% triglyceride, 11% egg lecithin), an effective substrate, decreases the rate of hydrolysis to about 50% that of the control.

Tests on the rates of hydrolysis after incorporation of the major components of plasma lipid into the soybean-oil emulsion have shown that cholesterol esters, phospholipids and free cholesterol all cause decreases. Phospholipids, and particularly cholesterol, were the most effective. Phospholipids in the substrate at concentrations of 20, 30 and 50% decreased the relative activity by 10, 60 and 80% respectively, whereas cholesterol at 2.5, 5, 10 and 30% caused decreases of 30, 40, 80 and 95% respectively.

In similar studies, increasing the amount of incorporation of phospholipids at fixed concentra-

tions of cholesterol caused very marked changes in the rate of hydrolysis of soybean oil. At low concentrations of cholesterol (5%) an optimum activity occurred in the presence of 20% phospholipid. With 15% cholesterol the optimum concentration of phospholipid was 30%, and at 30% cholesterol the optimum concentration of phospholipid was 50%. The relative activity at these optimum phospholipid concentrations was 65, 100 and 70% respectively.

These results show that the lipid composition of the substrate emulsion has a very marked effect on the activity of lipoprotein lipase. It is also known that surface-active agents used in the preparation of emulsions influence the activity (Fielding, 1970a; Doizaki & Zieve, 1968), but it is not yet known whether changes in plasma-lipid composition affect the tissue uptake of circulating triglyceride. The results of these experiments *in vitro* would suggest that the cholesterol concentration and the relative composition of the plasma lipids could be of importance in the activity of lipoprotein lipase *in vivo*.

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Dietary Alterations in Polyunsaturated Fatty Acids in Guinea-Pig Brain, Liver and Muscle

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Human cardiomyopathies are more commonly found in low socio-economic groups in parts of the world where the incidence of protein malnutrition is high (Shaper & Coles, 1965; Crawford, Gale, Somers & Hansen, 1970). The response of the endocardium and myocardium to low-protein diets has been demonstrated experimentally (Spatz, 1965, 1969; McKinney & Crawford, 1965; Reid & Berjack, 1966). In relating these findings to the human situation it became apparent that a food selection pattern leading to protein malnutrition is also likely to be poor in essential fats. Protein malnutrition arises through the selection of vegetable foods rich in carbohydrate; these are also poor in proteins and essential fats. Seeds, which provide the richest vegetable sources of protein and essential fats, also contain tocopherols. Consequently a diet

deficient in the essential fats is likely to be deficient in tocopherol, and low vitamin E concentrations have been recorded in the blood of communities using low-protein diets (Tulloch & Sood, 1967). Groups of six guinea pigs have been fed on diets deficient in the essential fats and tocopherols. Liver, muscle and brain were analysed and no differences could be found in the constituent phospholipid groups such as ethanolamine, choline and serine phosphatides. On the other hand significant alterations occurred in the fatty acids used for their construction. In the liver, docosa-hexaenoate was low ($1.1 \pm 0.3\%$, cf. $6.0 \pm 0.7\%$) and there was an increase in the docosa-, tri-, tetra- and penta-enoate contents. Eicosatetraenoate content ($5.5 \pm 0.7\%$) was less than in controls ($6.3 \pm 0.5\%$), but this was small compared with the fall in docosa-hexaenoate ($C_{22:6, n-3}$) content. The eicosatrienoate ($C_{20:3, n-9}$) content increased in a manner characteristic of fat deficiency.

Linoleate and linolenate contents also fell in liver and muscle, but there were still significant proportions of linoleate (8–15%) despite ataxia, other clinical symptoms and the severe changes in the longer-chain acids. In brain neither linoleate nor linolenate occurs in significant amount, and consequently changes were most readily seen in the long-chain acids, again with a loss of docosa-hexaenoate, the proportion of which dropped by one-third. This change was again accompanied by relative increases in docosa-, tri-, tetra- and penta-enoate contents.

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Plasma Structural Lipids in Groups at High and Low Risk to Atherosclerosis

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According to Walker (1963) coronary heart disease was the cause of death in 42.4% of white and 3.6% of Bantu males in the 55–64-year age groups in Johannesburg; epidemiological evidence and the international surveys (Kyu Taik Lee, Daoud, Jarmolych, Jakovic & Florentin, 1964; Scott, Likimani, Morrison, Thuku & Thomas, 1963)