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Fucose in Urine of Fasting Human Subjects

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By quantitative t.l.c. (Bell & Talukder, 1970) we observed that urines from fasting (12–15h) human volunteers invariably contained, besides galactose, glucose and fructose, the additional free monosaccharides ribose, xylose and arabinose. In experiments using a differential stain to reveal the sugar spots we found that fucose occupied the same chromatographic position as xylose. We could find no evidence of any chromatographic system in which fucose did not coincide with at least one of the accompanying urinary sugars (cf. Partridge, 1948).

One of us (M.Q.-K.T.) has now devised a rapid (65min) t.l.c. system that clearly separates fucose (R_F 0.72) from all other monosaccharides of 'fasting' urine (R_F 0.55 to 0.10); the method can be used quantitatively over the range 5–80 μ g. The plates are coated with Kieselguhr G (Merck) buffered with 0.15M- NaH_2PO_4 and developed with a mixture of ethyl acetate, methanol, butan-1-ol and water (16:3:3:2, by vol.). Sugars are revealed by spraying with aq. 90% acetic acid containing 1g of 4-aminobenzoic acid, 0.6g of sulphosalicylic acid (Bell, 1966) and 2g of SnCl_2 . After heating at 110°C for 20min hexoses show up yellow and pentoses pink, enabling separation to be confirmed. Areas containing the 6-deoxy sugars are scraped off, extracted with 90% acetic acid containing 2% (w/v) of SnCl_2 and the extinction read at 370nm, when a stoichiometric relationship holds between E 0.04 (5 μ g) and 0.6 (80 μ g) for each sugar.

Results from normal subjects and some pathological subjects will be presented.

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The Metabolism by Rats of Phenylmercury Acetate

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We have previously shown (Daniel & Gage, 1969; Daniel, Gage & Lefèvre, 1971) that methoxyethylmercury salts are rapidly degraded by the rat to yield mainly ethylene and inorganic mercury. This is probably connected with their lability *in vitro* in the presence of acid and cysteine (Weiner, Levy & Mudge, 1962). Phenylmercury salts are stable under these conditions, yet they are known to be rapidly metabolized in the rat to yield inorganic mercury (Gage, 1964).

The metabolism of phenylmercury in the rat has been studied after the subcutaneous administration of [^{14}C]phenylmercury acetate. About 85% of the radioactivity appeared in urine within 4 days and about 5% in the breath. Most of the mercury (50–60%) was excreted in faeces, with about 12% in urine.

About 2.5% of the dose appeared in urine as unchanged phenylmercury. Most of the radioactivity in urine was associated with sulphate and glucuronic acid conjugates of phenol. It seems likely therefore that phenylmercury is hydroxylated before cleavage of the carbon-mercury bond; if breakdown before hydroxylation occurred it is probable that substantial amounts of benzene would have been detected in expired air. Hydroxyphenylmercury salts are unstable *in vitro* in acid-cysteine, so it is possible that the liberation of inorganic mercury *in vivo* is not catalysed by an enzyme system.

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C₂₉-Carboxylic Acid Intermediates of Cholesterol Biosynthesis in Rat Liver

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Cholesterol biosynthesis from lanosterol in liver involves oxidative demethylation at C-14 and C-4 for which the sequence $\text{RCH}_3 \rightarrow \text{RCH}_2\text{OH} \rightarrow \text{RCHO} \rightarrow \text{RCOOH} \rightarrow \text{RH} + \text{CO}_2$ has been suggested (Olson, Lindberg & Bloch, 1957). Carboxylic acids are the only intermediates in this demethylation sequence to have been trapped (Miller & Gaylor,

1970; Hornby & Boyd, 1970). Certain C₂₈ and C₃₀ substrates give C₂₈ and C₂₉ acids respectively under conditions that prevent oxidation at C-3. Thus the formation of a 3 β -hydroxy-4 β -methyl-5 α -cholestene-4 α -carboxylic acid from lanost-8-enol has been reported (Hornby & Boyd, 1970). The double bond in this acid can now be located at Δ^8 .

A microsomal acetone-dried powder and a 105000g supernatant were prepared from rat liver as previously described (Hornby & Boyd, 1970; Scallen, Dean & Schuster, 1968). Each incubation mixture (4.5ml) comprised a suspension of 50mg of acetone-dried powder in 4ml of 105000g supernatant supplemented with glucose 6-phosphate (2.2mm), NADP⁺ (0.22mm), glucose 6-phosphate dehydrogenase (0.2 unit) and glutathione (4.3mm). No NAD⁺ was added. After 1 h at 37°C incubations were terminated with methanol. Carboxylic acids were recovered from the lipid product by t.l.c.

[2-³H₂]-4,4-Dimethyl-5 α -cholesta-8,14-dienol was synthesized (Klein & Knight, 1965) and incubated under these conditions to give the same acid as that obtained from lanost-8-enol, as judged by gas chromatography-mass spectrometry of their trimethylsilyl ether methyl esters. This acid cannot be $\Delta^{8(14)}$, since $\Delta^{8(14)}$ -monoenes, if involved in the biosynthetic pathway, would precede $\Delta^{8,14}$ -dienes. Unlabelled 4,4-dimethyl-5 α -cholest-7-enol gives 3 β -hydroxy-4 β -methyl-5 α -cholest-7-ene-4 α -carboxylic acid (J. L. Gaylor, personal communication), which has different gas chromatography-mass spectrometry characteristics. The compound from lanost-8-enol must therefore be 3 β -hydroxy-4 β -methyl-5 α -cholest-8-ene-4 α -carboxylic acid. The Δ^8 acids contained no Δ^7 acid by gas chromatography, which suggests that the $\Delta^8 \rightarrow \Delta^7$ isomerase is unable to accommodate a substrate with C-4 α carboxyl and C-4 β methyl groups. 4,4-Dimethyl compounds can be isomerized when anaerobic conditions prevent further demethylation (Gaylor, 1966). During cholesterol biosynthesis in rat liver, therefore, the $\Delta^8 \rightarrow \Delta^7$ isomerization seems to follow the loss of the C-14 and one of the C-4 methyl groups.

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The Effect of Dietary Cholesterol on Cholesterol Biosynthesis and other Processes in Mammalian Liver

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Cholesterol biosynthesis in mammalian liver is stimulated by injection of thyroid hormone (Guder, Nolte & Wieland, 1968) or phenobarbital (Jones & Armstrong, 1965); it is influenced by the concentration of dietary cholesterol and is depressed during starvation (Bucher, 1959). There is a diurnal variation in the rate of cholesterogenesis between a maximum at midnight and a minimum at midday (Hamprecht, Nüssler & Lynen, 1969). There is good evidence that the rate of cholesterol biosynthesis is dictated by the activities of those enzymes converting acetate into mevalonate; hydroxymethylglutaryl-CoA reductase (EC 1.1.1.34) in particular is stimulated or depressed by procedures that respectively accelerate or retard cholesterogenesis. There is little indication of how the activity of this enzyme is controlled.

Hydroxymethylglutaryl-CoA reductase is a microsomal enzyme and many of the factors affecting its activity are not specific in that they cause a variety of changes in the endoplasmic reticulum. For example, thyroid hormones are known to induce growth of the endoplasmic reticulum (Tata, 1968) and their effect of stimulating the reductase is one of the consequences. It is important that this should be so, because only by stimulating the reductase will increased production of cholesterol, necessary to sustain membrane proliferation, be accomplished. Indeed it is highly significant that the enzymes responsible for synthesizing other structural lipids, such as phospholipids, are themselves constituents of the microsomal membrane (Wilgram & Kennedy, 1963); in consequence the co-ordinated production of lipids and proteins, characteristic of growth, can be achieved. The action of other factors affecting hydroxymethylglutaryl-CoA reductase will be discussed in relation to their effects on other aspects of endoplasmic reticular metabolism.

During studies of the mode of action of cholesterol on hepatic cholesterogenesis, the influence of dietary cholesterol on certain microsomal functions has been investigated in the rat. The effect of dietary cholesterol on the rate of cholesterol biosynthesis is found not to be accompanied by changes in constitutive microsomal enzymes such as glucose 6-phosphatase or NADPH-cytochrome *c* reductase, or substances such as cytochrome *b*₅. The action of dietary cholesterol therefore appears to be rather specific in nature and the significance of these