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Hydroxylation of Fatty Acids by Avocado Supernatant Fraction

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Preparations from the avocado (*Persea americana*) mesocarp rapidly synthesize fatty acids from C₂ precursors. Yang & Stumpf (1965) first showed the incorporation of radioactivity from [¹⁴C]malonate and [¹⁴C]malonyl-CoA into long-chain fatty acids by the 15000g supernatant. This latter activity is due to soluble proteins rather than microsomal particles. However, the 105000g supernatant is unable to elongate medium-chain fatty acids (C₈-C₁₂) in the presence of added malonyl-CoA and appropriate cofactors (NADPH, acyl-carrier protein, GSH).

When fatty acids (C₈-C₁₂) were incubated with the 105000g supernatant under conditions used for fatty acid synthesis (Harwood & Stumpf, 1971) a hydroxy acid was produced. The possibility of the hydroxy fatty acid being α -hydroxy or ω -hydroxy was eliminated by chemical degradation and chromatographic analysis. The product co-chromatographed as the methyl ester or trimethylsilyl ether on g.l.c. and t.l.c. in two separate systems with the β -hydroxy derivative of the substrate. Butyrate, heptanoate, myristate, palmitate and stearate are not hydroxylated.

The reaction requires CoA, ATP, acyl-carrier protein and molecular O₂. The chain-length specificity is not due to the acid-CoA ligase, since myristyl-CoA is not hydroxylated. It does not take place via β -oxidation because (a) no radioactivity was incorporated from ³H₂O under the reaction conditions, (b) no loss of radioactivity from 1-¹⁴C-labelled medium-chain fatty acids took place during the incubation and (c) the observed specificity would not have occurred since β -oxidation utilizes long-chain acids as well.

The hydroxylating enzyme is sensitive to iodoacetamide and, consequently, is protected by GSH. Production of the β -hydroxy acid is diminished in the presence of NADH and/or malonyl-CoA. This is probably due to the production of NAD⁺, since

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addition of the latter (at 0.5mM) completely inhibits the reaction. Slight inhibition of the reaction was achieved with cyanide (0.1mM); 5,5'-bipyridyl (1 μ M) or CO (CO+O₂, 83:17) gave strong inhibition, thus suggesting that a heavy-metal ion (such as Fe²⁺) is involved in the hydroxylation. Because of the soluble nature of the avocado hydroxylating system it should be possible to purify the enzyme and study the exact mechanism of hydroxylation.

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Low-Fat Diets and the Survival of Newborn Rats

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Phospholipids and protein are the major components of cellular and subcellular membranes. In animal tissues the essential fatty acids and their long-chain metabolic derivatives are consistently found as the major fatty acids in phospholipids.

In the rat essential-fatty acid deficiency affects reproduction: deficient females frequently abort or the pups die soon after birth; the male rats are generally sterile and will not mate (Holman, 1968). Deficiency experiments are frequently carried out by using diets completely free from fat; however, this is a situation most unlikely to occur in Nature. We examined rats fed on a low-fat diet (0.6% fat; 10g of diet contained 11mg and 2.2mg of linoleate and linolenate respectively) from weaning to maturity; on this diet the rats did not develop markedly scaly tails and the eicosatrienoate/arachidonate ratio in their liver phospholipids was only 0.1. Female rats on this diet were mated and only about 12% of the pups (13 litters) survived longer than 2 weeks. The same diet with 6% fat added isocalorically (10g of diet contained 211mg and 43mg of linoleate and linolenate respectively; control diet) gave a survival rate greater than 50% (12 litters).

Tissues were analysed within 24h of birth from 11 randomly selected pups on the low-fat diet and nine on the control diet. On the low-fat diet the pups were 27% smaller and there was significantly less lipid in their livers (24.3 $\pm$ 2.3mg/g compared with 45.6 $\pm$ 4.7mg/g). The percentage of *n*-6 and *n*-3 unsaturated fatty acids in the liver lipids was decreased by 50% and the eicosatrienoate/arachidonate ratio was 0.47. The pups of the mothers given this diet had smaller brains (0.20 $\pm$ 0.01g compared with 0.29 $\pm$ 0.01g) and the polyenoic fatty acid content was decreased by 20%. The fatty acid composition had not altered as

much as in the liver. The stomach content (milk) lipids contained significantly less of both the essential fatty acids and their longer-chain metabolic derivatives.

In the liver lipids of rats fed on completely fat-free diets eicosatrienoate/arachidonate ratios greater than 3 have been reported (Holman, 1968). Our results show that considerable morphological changes took place in these pups before this characteristic fatty acid pattern became established.

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Urinary Sugar Excretion Related to Lactose and Sucrose Intake in Normal Children

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The excretion of disaccharides in the urine of children suffering from certain gastrointestinal disorders has been recognized for many years (Bickel, 1961; Gryboski *et al.*, 1963). More recently, interest in this phenomenon has been increased by the observation that it occurs particularly when intestinal disaccharide hydrolysis is impaired. Several authors have emphasized the relationship between excretion and intake of sugar (Folin & Berglund, 1922; Utter, 1927; Haworth, 1960). The data at present available are difficult to evaluate because test conditions and the presentation of results vary considerably from author to author.

Disaccharide loading tests were successfully completed on 300 'normal' children aged between 2.5 and 13 years and having a normal weight distribution (11–55 kg) who were awaiting tonsillectomy. A baseline specimen (early morning) and all the urine passed in the 5 h period after the load were collected and the sugars were determined quantitatively by paper chromatography (Menzies & Seakins, 1969). The period of collection was based on the observations made by Utter (1927) and McCance & Madders (1930).

In the first group, when the concentration of lactose or sucrose (25–40 g) was not controlled 60% of the children failed to excrete either disaccharide, and 50% either fructose or galactose. When the two disaccharides were combined (20 g each) none of the subjects excreted either, but when the load was increased (30 g each) 20% of the subjects excreted the disaccharides.

In the second group, when the concentration of sucrose (30 g) was varied between 20 and 70 g/100 ml sucrose excretion increased with increasing concentration, particularly in the range 50–70 g/100 ml.

Except at the lowest end of the quantity-concentration range, the administration of lactose and sucrose (20 or 30 g each), mixed in the same solution in concentrations of 20, 25 and 30 g/100 ml, produced an increase in the mean excretion of both disaccharides compared with that given by either disaccharide alone in the same amount and concentration. In two-thirds of the subjects sucrose excretion exceeded that of lactose by up to 400 mg/5 h.

These results confirm the pioneer work of Utter (1927) on the effect of disaccharide concentration and extend Moncrieff's (1960) observations on the administration of mixtures of disaccharides. Despite statements to the contrary (Lindemann & Solomon, 1962), lactose and sucrose can cross the mucous membranes of the normal gastrointestinal tract.

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Alimentary Disacchariduria in Adults Related to the Osmolality of Ingested Solutions

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The observation that disacchariduria in children can be increased by addition of other sugars to an oral disaccharide load (Moncrieff, 1960; Menzies & Seakins, 1969) may indicate the effect of mechanisms relevant to the interpretation of disacchariduria, especially in gastrointestinal disease.

Factors influencing alimentary disacchariduria were studied by observing the effects of varying the disaccharide quantity and concentration in, and addition of other sugars and solutes to, oral loads in a group of normal starving adults. Quantitative determination of sugars in urine was performed by paper chromatography and direct scanning (I. S. Menzies, unpublished work).

As with children, admixture of sucrose and lactose, when raised above a certain concentration (40 g/133 ml each), progressively increased the 5 h urinary excretion of both disaccharides compared with that after either disaccharide given alone in the same amount and concentration.