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Hormone-Receptor Interaction as a Guide to Biochemical Mechanism

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COMMUNICATIONS

Effect of Bile and Pancreatic Juice on the Absorption of Long-Chain Fatty Acids in the Sheep

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In further investigations of the role of pancreatic juice in the digestion and absorption of fat in the ruminant we have studied the absorption into the lymphatic circulation of radioactively labelled stearic acid, palmitic acid and oleic acid. Sheep were suitably prepared with a thoracic-duct fistula and with an intestinal cannula which allowed diversion of the mixed secretions of bile and pancreatic juice. When bile and pancreatic juice flowed normally into the intestine the recovery in the lymph of stearic acid, palmitic acid and oleic acid was 54, 69 and 83% respectively of the radioactivity introduced into the duodenum; 85-95% of the radioactivity was located in the triglycerides. As in non-ruminants, therefore, the absorption of long-chain fatty acids increases in the order stearic acid < palmitic acid < oleic acid (Deuel, 1954).

When the mixed secretions of bile and pancreatic juice were diverted from the intestine, and bile alone infused, the absorption of stearic acid, palmitic acid and oleic acid fell to 0.8, 4.2 and 24.2% respectively of the dose infused. When pancreatic juice alone was infused, with diversion of mixed secretions, less than 1% of the dose was recovered in the thoracic-duct lymph. These results vindicate the conclusions of Heath & Morris (1964) that both bile and pancreatic juice are required for optimum fat absorption in the sheep.

Other experiments involving incubation of

intestinal segments *in vitro* and observations on intestinal loops *in vivo* have shown that, in the absence of pancreatic juice, fatty acids in a micellar form with bile enter the mucosal cell and are re-synthesized rapidly into triglycerides. It appears likely, therefore, that pancreatic juice functions at the luminal stage of digestion, probably by releasing fatty acids from food particles and allowing their solubilization in bile salts; the lysolecithin formed from bile by pancreatic juice may be partly or completely responsible for this action (Leat & Harrison, 1969).

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Base Exchange Reactions of Phospholipid Metabolism in Chick Brain Microsomal Membranes

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The incorporation of nitrogenous bases into phospholipid follows at least two distinct patterns in animal tissue. The first involves the classical Kennedy's pathway and depends on cytidine nucleotides and Mg^{2+} . Its operational existence in nervous tissue has been adequately demonstrated (see Porcellati, Biasion & Pirotta, 1970). The

second is represented by Ca^{2+} -stimulated, energy-independent exchange reactions of serine, ethanolamine and other amino alcohols with endogenous phospholipids. Preliminary reports have indicated that these reactions could take place in brain tissue (McMurray, 1964; Porcellati, di Jeso & Malcovati, 1966; Ansell & Spanner, 1966; Pirotta, Giorgini & Porcellati, 1969).

A Ca^{2+} -stimulated enzymic system has been detected in purified brain microsomal membranes, which exchanges with endogenous phospholipid bases about 10% of the incubated L-[3- ^{14}C]serine after 30–60 min of incubation. About 90% of this radioactivity is confined to phosphatidylserine. [1,2- ^{14}C]ethanolamine is also incorporated into microsomal phosphatidylethanolamine (60–80 nmol/h per mg of protein), whereas no incorporation of choline occurs by similar mechanisms. The transfer of ethanolamine cannot be decreased by adding unlabelled phosphorylethanolamine, indicating that the reaction does not represent a net synthesis of phospholipid. Mitochondria and high-speed supernatants are almost inactive in exchanging phospholipid bases.

The exchange reaction requires Ca^{2+} , with maximal activity at 25–30 mM, does not take place if Mg^{2+} or detergents replace Ca^{2+} , and is still efficient if both Ca^{2+} and Mg^{2+} are present in the medium. The transfer of ethanolamine and serine to lipid is abolished by EDTA, depressed by Mn^{2+} , Hg^{2+} , Co^{2+} and Cu^{2+} , and only slightly inhibited by 20 mM-tris.

The serine-exchange system is apparently saturated by 2.4 mM-serine, but a noticeable decrease in reaction rates occurs with increasing substrate concentration. Labelled serine is competitively displaced from the enzymic site by 1 mM unlabelled ethanolamine, the reverse also being true. The presence of endogenous phospholipids in the system certainly interferes with the carrying out of correct kinetic studies. Active fractions were therefore prepared from the microsomal membranes, by use of cross-reactions with detergents and ammonium sulphate, which are fairly devoid of phospholipids. Further improvements need, however, to be developed, because of the low yield of activity obtained by these treatments.

The enzymic system reported in this work can be probably compared with those described for liver tissue (Borkenhagen, Kennedy & Fielding, 1961; Hübscher, 1962) and insect fat-bodies (Crone, 1967).

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Chain-Length Specificity of Inhibition of Fatty Acid Oxidation by Pent-4-enoyl-L-Carnitine and by Pent-4-enoic Acid

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Pent-4-enoic acid (0.01–0.1 mM) strongly inhibits formation of $^{14}\text{CO}_2$ from 1- ^{14}C -labelled stearate, palmitate and myristate, but not from fatty acids with shorter chain lengths, in rat liver mitochondria when incubated in a medium containing 1.0 mM-DL-carnitine and 0.8 mM-fumarate in the Warburg apparatus (Senior, Robson & Sherratt, 1968). Polarographic measurements showed that inhibition of palmitate oxidation is abolished under uncoupling conditions, suggesting that it is caused by intramitochondrial pent-4-enoyl-CoA (and/or some of its β -oxidation products). Additional evidence was that pent-4-enoyl-L-carnitine strongly inhibited palmitoyl-L-carnitine oxidation in uncoupled mitochondria (Holland & Sherratt, 1969).

We now report the effects of 0.1 mM-pent-4-enoyl-L-carnitine on the oxidation of stearoyl-, palmitoyl-, myristoyl-, lauroyl-, decanoyl-, octanoyl-, hexanoyl- and butyryl-L-carnitine, measured polarographically under uncoupling conditions in the presence of 20 mM-malonate. It was found unexpectedly that all oxidations were strongly inhibited (70–80%). A 3 min preincubation with pent-4-enoyl-L-carnitine was necessary; if this was added while acylcarnitines were being oxidized rapidly no inhibitions were found. Similarly, 0.1 mM-pent-4-enoic acid inhibited the oxidation of palmitoyl- and octanoyl-L-carnitine in coupled mitochondria by 70–80%. These results suggest that there is no inhibition of carnitine acetyltransferase or of carnitine palmitoyltransferase by pent-4-enoic acid derivatives, and this was confirmed with preparations of these enzymes, pent-4-enoyl-CoA and pent-4-enoyl-L-carnitine being substrates for carnitine acetyltransferase (P. C. Holland, A. E. Senior & H. S. A. Sherratt, unpublished work).

The apparent chain-length specificity of inhibition of fatty acid oxidation found by Senior *et al.* (1968) could be related to the high concentrations of substrate (usually 1.0 mM) necessary with relatively long manometric experiments. Long-chain fatty acids are thought to be activated in an outer mitochondrial compartment (outside the 'carnitine