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Evidence for the Existence of an Extramitochondrial Pathway of Acetoacetate Synthesis in Rat Liver

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Key enzymes involved in hepatic ketogenesis, 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) synthase (EC 4.1.3.5) and HMG-CoA lyase (EC 4.1.3.4) have been assayed to test whether the increase in ketone-body concentrations in starvation and diabetes are accompanied by increased enzyme capacities. The liver was homogenized with 0·25M-sucrose containing 10mM-tris, pH 7·4 and 1mM-mercaptoethanol, and then fractionated according to the method of Streffer & Williamson (1965). The whole homogenate and particulate fraction were treated sonically before determination of enzyme activity. HMG-CoA synthase activity was measured by incubation of liver fractions with an acetyl-CoA generating system (Lynen, Henning, Bublitz, Sorbo & Kröplin-Rueff, 1958) and HMG-CoA lyase by incubation with excess HMG-CoA. The acetoacetate formed was subsequently determined enzymically (Williamson, Mellanby & Krebs, 1962).

In normal rat liver the *total* activity for HMG-CoA synthase was found to be 18 ± 6 μmoles/min./100g. body wt. (mean value ± S.D., 6 animals); for HMG-CoA lyase, 41 ± 8 μmoles/min./100g. body wt. (6 animals). There were no appreciable differences in the total HMG-CoA synthase and lyase activities of livers from normal, starved, alloxan-diabetic and fat-fed rats, especially when expressed on a units/100g. body wt. basis. Thus changes in the total activities of these enzymes are not responsible for the ketosis of starvation and alloxan-diabetes.

The cytoplasmic activity of the two enzymes in normal liver was less than 10% of the total activity, but this extramitochondrial activity increased at least twofold in livers from starved and alloxan-diabetic rats. With fat-fed rats the increase was less. A similar increase in cytoplasmic activity of HMG-CoA synthase has been reported in livers of ketotic guinea pigs (Sauer & Erfle, 1966). On refeeding a normal diet to rats previously starved for 48hr. the proportion of the cytoplasmic HMG-CoA synthase and lyase activities fell to normal levels within 24hr.

These changes in the cytoplasmic activity of the HMG-CoA pathway enzymes are not due to increased mitochondrial leakage, because there were no significant changes in the cytoplasmic activity of glutamate dehydrogenase (a mitochondrial marker).

Approximately 22% of the total acetoacetyl-CoA thiolase activity of rat liver was found in the cytoplasm, and therefore all the enzymes necessary for acetoacetate synthesis from acetyl-CoA are present outside the mitochondria. The role of this extramitochondrial pathway might be to dispose of excess acetyl-CoA arising in the cytoplasm when lipogenesis is depressed as in starvation and alloxan-diabetes.

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Mode of Uptake of Triglyceride by the Goat Mammary Gland

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The uptake of plasma triglyceride calculated from arteriovenous difference studies and rates of blood flow can account for about 60% of milk fat production (Annison, Linzell, Fazakerley & Nichols, 1967). Several groups of workers have shown that both chylomicron fat and plasma β-lipoproteins are milk fat precursors but little is known of the mechanism of uptake, although the increased levels of lipoprotein lipase in mammary venous plasma observed by Barry, Bartley, Linzell & Robinson (1963) suggested that lipolysis might accompany triglyceride uptake. We have investigated the transfer of chylomicron fat into milk using doubly-labelled chylomicrons.

Intestinal lymph was collected from a donor goat during a duodenal infusion of [1-^{14}C]glycerol tripalmitate and [9,10-$^{3}H_2$]palmitic acid, and the chylomicrons were separated and washed by centrifugation. This material was continuously infused (70min.) into the mammary artery of a fed goat which was milked at 60min. intervals using oxytocin (200 milliunits) to aid milk ejection. Arterial and mammary venous blood samples were removed at 15min. intervals during and after the infusion, and lymph was collected continuously (Linzell, 1960) and examined at 30min. intervals. The uptake of chylomicron triglyceride relative to that of other β-lipoprotein triglycerides and of

plasma FFA, the release of FFA from chylomicrons, the appearance of labelled lipid in lymph and the incorporation of labelled fat into milk were measured. The following data were obtained: (1) A substantial decrease in the ratio $^{14}C/^{3}H$ in milk triglyceride relative to that of chylomicron triglyceride (1·0 : 0·65). (2) The absence of labelled glycerides (tri-, di- and mono-) in lymph. (3) The release of appreciable amounts (25%) of chylomicron triglyceride fatty acid into mammary venous plasma. (4) Lymph FFA and mammary venous FFA showed similar specific radioactivity.

These results are consistent with the hypothesis that substantial or complete hydrolysis of plasma triglyceride at the capillary cell precedes uptake by the mammary gland.

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Carotenoids and Fatty Acids as Uncouplers of Photophosphorylation in Isolated Chloroplasts

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Kahn & Purcell (1965) added carotenoids to isolated spinach chloroplasts and found that some of the pigments stimulated and others inhibited photosynthetic electron transport. In order to determine whether these effects were related to the chemical structure of the carotenoids, the effects of known xanthophylls, in particular antheraxanthin and lutein, on the Hill reaction and on photophosphorylation of chloroplasts isolated from broad bean (*Vicia faba* L. c.v. *Aquadulce*) have been examined. The Hill reaction measured as photoreduction of either potassium ferricyanide or 2,6-dichlorophenol-indophenol was stimulated by low concentrations and inhibited by higher concentrations of added carotenoids. In the presence of ammonium sulphate which accelerates the Hill reaction by its uncoupling action (Krogmann, Jagendorf & Avron, 1959), the carotenoids inhibited photoreductions of both acceptors at all concentrations tested. The photoreduction of ferricyanide in the presence of ADP, and the concurrent formation of ATP (Arnon, Whatley & Allen, 1958) were both inhibited by added carotenoids. Antheraxanthin was more effective than lutein in stimulating both nonphosphorylating Hill reactions and in inhibiting indophenol reduction in the presence of ammonium sulphate. However, lutein was more effective than antheraxanthin as an inhibitor of ferricyanide photo reduction in the presence of either ADP or ammonium sulphate.

The carotenoids therefore are acting both as uncouplers and as energy transfer inhibitors according to the criteria of Good, Izawa & Hind (1966) since they stimulate non-phosphorylating electron transport and inhibit photophosphorylation (uncoupling) and they also inhibit phosphorylating electron transport (inhibition of energy transfer).

The effects of added linolenic, linoleic and oleic acids on broad bean chloroplasts were similar to those of the carotenoids, and thus similar to the effects of linolenic acid on spinach chloroplasts found by McCarty & Jagendorf (1965). The major difference between the action of added carotenoids and of unsaturated fatty acids on broad bean chloroplasts is that for similar effects the molar concentration of fatty acid required is tenfold that of carotenoid.

Triton-X-100 has similar effects on spinach chloroplasts (Izawa & Good, 1965) and the similar effects of lipids and detergents may well be by interaction with the complex lipids of the grana membranes.

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Perfusion of Rat Liver with Adenosine Monophosphate

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Adenosine 5′-monophosphate is known to effect significant intracellular metabolic changes, especially in muscle. (Stadtman, 1966). In general an increased level of AMP appears to act as a signal for increased catabolic activity. We have perfused rat livers using the system of Mortimore (1961). Pairs of livers were perfused for one hr. one liver acting as control, the other being perfused with 3mM-AMP. Incorporation of [^{3}H]orotic acid into nucleic acids and [^{14}C]phenylalanine into proteins was assayed in starved rats. AMP inhibited the former by 60% and the latter by 20%. Assay of