of malonate and malonyl-CoA into fatty acids by the 15000 g supernatant of this homogenate.

Mitochondria prepared from the avocado mesocarp by the procedure of Baker, Elvin, Biale & Honda, (1968), in which the tissue is fragmented with a plastic vegetable grater and the mitochondria are separated by centrifuging on a sucrose density gradient, showed a high degree of respiratory control of the oxidation of tricarboxylic acid-cycle intermediates in the presence of ADP but did not incorporate [1-¹⁴C]acetate into fatty acids.

Although the supernatant did not incorporate [1-¹⁴C]acetate or [2-¹⁴C]malonate into fatty acids the green layer, more dense than the mitochondria on the sucrose density gradient, formed radioactive palmitate (45%) and oleate (45%) from [1-¹⁴C]acetate.

Chloroplasts obtained from a similar homogenate by sedimentation in 0.3 M-sucrose at 2000 g incorporated [1-¹⁴C]acetate into palmitate and oleate, but the supernatant again showed no incorporation of [1-¹⁴C]acetate or [2-¹⁴C]malonate into fatty acids.

Homogenates obtained by the procedure of Yang & Stumpf (1965) by using a Waring Blendor failed to give a chloroplast pellet on centrifugation at 2000 g in 0.3 M-sucrose. The 15000 g supernatant, however, incorporated [2-¹⁴C]malonate into stearate (60%) and oleate (37%); the incorporation that was obtained accounted for the total radioactivity of the parent homogenate when allowance was made for the volume.

When chloroplasts and mitochondria were sedimented together at 12000 g from a homogenate prepared by the method of Hobson, Lance, Young & Biale, (1966) the incorporation of [1-¹⁴C]acetate into fatty acids was the same as that given by chloroplasts sedimented at 2000 g from the same homogenate.

From this it is concluded that neither the cytoplasm nor the mitochondria of the avocado mesocarp synthesize fatty acids from acetate or malonate. Fatty acid synthesis from acetate occurs in the chloroplasts or other particles sedimenting therewith, and rupture of these particles during preparation gives rise to fatty acid synthesis apparently in the cytoplasm and mitochondrial fraction.

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Adipose-Tissue Lipoprotein Lipase Activity in Rats Adapted to Controlled Feeding Schedules

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The transition of low lipoprotein lipase activity of adipose tissue in starved rats to high activity in fed animals is dependent on carbohydrate metabolism and insulin. The opposite change from high to low activity is not perfectly understood. It is known that lipoprotein lipase activity decreases within a few hours after a fed animal is deprived of food (Wing & Robinson, 1968), probably before absorption of nutrients from the gastrointestinal tract ceases.

The mechanism underlying these changes cannot be studied in detail in the absence of further knowledge of the true course of these events. We have therefore investigated the activity of lipoprotein lipase in adipose tissue of rats adapted to food at fixed intervals. We used two groups of animals; one had access to food between 7 p.m. and 9 a.m. every day, the other between 7 p.m. and 9 a.m. every second day. Animals were killed at 3 h intervals and lipoprotein lipase activity was determined in the epididymal fat-pads. Lipoprotein lipase was assayed in ammonia extracts of acetone-dried powders from adipose tissue of individual rats by using an artificial substrate (Korn & Quigley, 1957). Free fatty acids were determined by the method described by Novak (1956), plasma glucose by the glucose oxidase method with reagents from C. F. Boehringer und Soehne (Mannheim, Germany) and plasma immunoreactive insulin by the method described by Samols & Bilkus (1964).

The changes in lipoprotein lipase activity followed a similar pattern in both groups of animals during the time in which food was available; activity increased steadily for approx. 10 h and then started to decline when the animals still had access to food.

When the animals no longer had access to food lipoprotein lipase activity continued to decline in the group adapted to having food each night. In intermittently starved animals this decline was moderate and was followed by a second peak of activity during the night when no food was available. No peak in plasma immunoreactive insulin or glucose, nor a change in plasma free fatty acids, was observed during the night. When actinomycin D (125 µg/rat), was given before the peak that occurred during the starvation period no increase in lipoprotein lipase activity was detected.

Our results show that the immediate cause for the decline of lipoprotein lipase activity in fed rats is not a decreased availability of nutrients. They

also show that the increase in lipoprotein lipase activity is not necessarily associated with an increase in plasma insulin concentrations.

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Interaction of Insulin with Phospholipid in a Membrane Model

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Insulin action has been suggested to involve binding of the hormone to protein receptors in the cell membrane, possibly by a disulphide-interchange reaction (Rieser, 1967). However, the possibility that insulin interacts with the phospholipids of cell membranes has received little attention. We are investigating the interaction of insulin with phospholipid model membrane systems.

The experimental system was modified from that of Jung, Chaney & LeFevre (1968); phosphatidylcholine and phosphatidic acid were prepared from fresh hen's eggs and purified by chromatography on silicic acid. Other phospholipids were obtained commercially. Chloroform (1ml) was incubated at 37°C for 72h under 1ml of an aqueous phase (125mM-sodium chloride, 5mM-potassium chloride, 35mM-tris-HCl buffer, pH 7.4) containing [14 C]glucose (0.3–1 μ Ci/ml). We have confirmed the observations of Jung *et al.* (1968) that phosphatidylcholine in the chloroform increased the amount of [14 C]glucose appearing in the organic phase during incubation, probably by the formation of a phosphatidylcholine-glucose complex. Insulin (4–40 μ g) in the aqueous phase decreased the amount of [14 C]glucose solubilized by phosphatidylcholine in the chloroform, but did not affect the solubilization in the absence of phosphatidylcholine. The distribution ratio of [14 C]glucose between the chloroform and aqueous phases reached equilibrium after incubation for 60h and remained constant for 200h. At all time-intervals tested, insulin decreased the amount of [14 C]glucose solubilized by the phosphatidylcholine.

A variety of lipids has been tested in this system. The order of effectiveness of the lipids at a concentration of 1mM in solubilizing [14 C]glucose in the chloroform phase was cholesterol < phosphatidylcholine < sphingomyelin < phosphatidylethanolamine < phosphatidic acid. Cholesterol did not

solubilize [14 C]glucose in chloroform. Insulin decreased the amount of [14 C]glucose solubilized by phosphatidylcholine, phosphatidylethanolamine and phosphatidic acid, but not by sphingomyelin.

The decrease in [14 C]glucose solubilization observed in the presence of insulin was not the result of a change in the phosphatidylcholine concentration in the chloroform phase. Measurement of the phospholipid (Baginski, Foa & Zak, 1967) in the two phases after incubation for 72h showed that there was no loss of phosphatidylcholine from the chloroform in the presence or absence of insulin.

It seems probable that insulin forms a complex with the phosphatidylcholine in chloroform resulting in displacement of [14 C]glucose from the phospholipid into the aqueous phase. Studies in progress with 125 I-labelled insulin support this hypothesis.

M. C. P. is a Medical Research Council Research Fellow.

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Post-Mortem Changes in Liver Microsomal Protein-Synthesizing Activity

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As a preliminary to studying post-mortem samples of human liver for various enzyme activities, it was thought necessary to get some measure of the liver microsomal amino acid-incorporating activity (Jondorf, Spector & Chaiken, 1965) in the rat at various times after death.

Groups of 160g female Sprague-Dawley rats were killed simultaneously by stunning and decapitation and were left at room temperature. Livers from pairs of rats were excised at zero time and at 1, 2, 4, 6 and 16h after death, and were then processed for the preparation of microsomal and 105000g-supernatant fractions under standard conditions (Jondorf, Simon & Avnimelech, 1966).

Endogenous mRNA-dependent L-[14 C]phenylalanine incorporation into protein diminished by about 35% in the first 1h and was only about 20% of the initial activity after 6h. At 16h, a certain amount of autolysis had taken place, and the yield of microsomal fraction per g of liver was only about 50% of the zero-time yield. These 16h microsomes still retained some marginal protein-synthesizing activity.

In experiments where the various microsomal preparations were incubated with zero-time