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.

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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