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

105000g-supernatant fraction in the system *in vitro*, the incorporation activities were very similar to those of incubation mixtures where microsomes were recombined with their own corresponding supernatant fractions. This suggested that the tRNA component of the system localized in the supernatant fraction is relatively stable.

By preincubating the microsomal system, the endogenous mRNA activity is lost and the microsomes become fully receptive to exogenous mRNA such as polyuridylic acid when appropriately reincubated (Kato, Jondorf, Loeb, Ben & Gelboin, 1966). The microsomal sensitivity to added mRNA decays rapidly and follows the post-mortem time-course for loss of endogenous mRNA-dependent protein-synthesizing activity.

The loss of microsomal protein-synthesizing capacity at 6h *post mortem* is greatly diminished if the rat carcasses are kept at 0–4°C. With livers excised at zero time and kept at 0–4°C for 6h before processing, the loss of endogenous mRNA- and polyuridylic acid-directed phenylalanine incorporation is only 20–30%.

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ω -Oxidation of S-Pentyl-L-cysteine

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S-Alkylcysteines are converted into a variety of products in the animal body. Deamination may occur and a group of compounds arising from the alkylthioketo acids formed have been described (Barnsley, 1964; Sklan & Barnsley, 1968; Harris, James & Needham, 1968). Acetylation to the corresponding mercapturic acid is a major metabolic pathway and may be accompanied by S-oxidation to give mercapturic acid sulfoxides (Barnsley, Eskin, James & Waring, 1969). Oxidation of the alkyl chain leading to the formation of hydroxy-alkylmercapturic acids has been described for butylcysteine (Barnsley *et al.* 1969). A further oxidation product has now been detected in which the terminal methyl of the pentyl chain is oxidized to a carboxyl group.

When pentylcysteine was given to rats a metabolite was formed which was shown, by its chromatographic and electrophoretic behaviour, to be more acidic than pentylmercapturic acid. The metabolite was separated by paper chromatography

and converted into its methyl ester. The product gave only a single peak on g.l.c. The mass spectrum of the ester showed a peak at *m/e* 291 corresponding to the dimethyl ester of *N*-acetyl-S-(4-carboxybutyl)cysteine, $C_{12}H_{21}NO_5S$; further peaks at *m/e* 232 and 190 corresponding to the loss of $C_2H_3O_2$ and $C_3H_3O_2$ were present. Mass measurement of the peaks at *m/e* 291 and 232 supported the assigned formulae. The mass spectrum of the unesterified metabolite showed peaks at *m/e* 218 and 176 corresponding to the loss of CO_2H and $C_4H_7O_2$ from the molecular ion $C_{10}H_{17}NO_5S$.

The methyl ester of the metabolite was identical in chromatographic properties with the methyl ester of the synthetic compound prepared by the interaction of 5-bromopentanoic acid and *N*-acetylcysteine.

Rabbits and mice dosed with pentylcysteine also excreted *N*-acetyl-S-(4-carboxybutyl)-L-cysteine and the metabolite was identified in the urine of rats dosed with 5-bromopentanoic acid, 1,5-dibromopentane or 1-bromopentane. It was also detected in the urine of rabbits dosed with bromopentane and was formed on incubation of pentylcysteine with rabbit liver slices.

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Hydroxylation of 1-Bromopentane Involving Cytochrome P-450

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The formation of alkyl- and hydroxyalkylmercapturic acids as metabolites of 1-bromopropane and 1-bromobutane has been described (Barnsley, 1964; James, Jeffery, Waring & Wood, 1968). It is now shown that, in addition to pentylmercapturic acid, 1-bromopentane gives rise in the rat to several metabolites which are derivatives of pentylmercapturic acid in which the pentyl chain is oxidized. These metabolites were separated from the urine of dosed rats by adsorption on charcoal at pH 4.5 or by ether extraction, and were treated with diazomethane to give methyl esters, which were examined by g.l.c. The presence of the methyl esters of 2-hydroxy- and 3-hydroxy-pentylmercapturic acid was demonstrated by comparison with the methyl esters of synthetic samples of these acids. Chemical hydroxylation of pentylmercapturic acid by the method of Udenfriend, Clark, Axelrod & Brodie (1954) resulted in the