PROCEEDINGS OF THE BIOCHEMICAL SOCIETY

The 496th Meeting of the Society was held at the University of Oxford on Thursday and Friday, 10 and 11 July 1969, when the following papers were presented:

LIPID GROUP COLLOQUIUM ON 'FATTY ACID TRANSPORT'

Fatty Acid Mobilization in Adipose Tissue, with Particular Reference to the Effects of this Process on Fat-Cell Metabolism

By B. JEANRENAUD. (*Institut de Biochimie Clinique, University of Geneva Medical School, Geneva, Switzerland*)

(1) Fat cells are known to be influenced by a number of hormones that stimulate fatty acid mobilization. These hormones have been conveniently divided into two groups, the fast-acting hormones (e.g. adrenaline), and the delayed-type FFA*-mobilizing hormones (e.g. glucocorticoid and growth hormones). The main characteristics of these two groups are as follows. The first group produces a rapid increase in the concentration of cyclic AMP that is mediated through activation of adenylyl cyclase. The second group either inhibits glucose entry, with a consequent decrease in fatty acid re-esterification and hence an increased FFA output, or stimulates RNA synthesis and hence the synthesis of specific proteins involved in the regulation of lipolysis.

(2) The finding that ouabain addition *in vitro* inhibits the lipolytic activity brought about by the fast-acting lipolytic hormones (adrenocorticotrophic hormone, adrenaline, glucagon) suggests a relationship between the lipolytic process and Na^+ - K^+ -pump activity. The effect of ouabain can be mimicked by removing K^+ from the medium, indicating that inhibition of cationic transport may be the primary effect of this agent. In addition, the inhibitory effect of ouabain, or of K^+ lack, on hormone-induced lipolysis occurs in the absence of glucose, suggesting that the effect may be exerted at the level of adenylyl cyclase. Indeed, preincubation of adipose tissue with ouabain, or in the absence of K^+ , decreases adenylyl cyclase activity by 60–70%, whereas phosphodiesterase activity is unaffected.

(3) Increased lipolytic activity in adipose tissue results in an increase in intracellular FFA concentrations. These acids may be an important factor regulating the overall metabolism in fat cells. They seem to influence glucose metabolism, as well as K^+ and amino acid uptake in the cells.

* Abbreviation: FFA, free fatty acids.

(4) All agents or conditions that may be expected to decrease intracellular concentrations of FFA in adipose tissue exert similar effects on glucose metabolism by fat cells. These effects are, principally, an increase of glucose metabolism through the pentose cycle and an increase in long-chain fatty acid synthesis from glucose. They can be mimicked by decreasing FFA concentrations in the medium. On the other hand, although lipolytic agents also increase glucose uptake (as does an increase in the concentrations of FFA in the medium), the pattern of glucose metabolism is mainly characterized by a decrease in glucose metabolism through the pentose cycle and a decrease in long-chain fatty acid synthesis from glucose. Possible mechanisms of the effects of FFA on glucose metabolism will be discussed.

(5) It has been observed that K^+ and α -aminoisobutyric acid accumulation by isolated fat cells is energy-dependent. Fast-acting lipolytic agents (such as adrenocorticotrophic hormone or adrenaline) markedly inhibit both K^+ and α -aminoisobutyric acid accumulation. Further, an inverse relationship was observed between lipolytic activity and K^+ or α -aminoisobutyric acid uptake, suggesting that, when active lipolysis takes place (with the possible accumulation of FFA or other products of lipolysis within the cell), energy availability was decreased. In the presence of lipolytic hormones, insulin restored K^+ or α -aminoisobutyric acid uptake towards normal, exactly as it inhibited lipolysis, suggesting that energy availability is again likely to be the key. These inhibitory effects on K^+ or α -aminoisobutyric acid accumulation could be mimicked by increasing FFA concentrations in the medium.

(6) The data on K^+ and α -aminoisobutyric acid uptake suggest that several metabolic effects brought about by lipolytic or anti-lipolytic agents may be related to modifications of the energy economy of the fat cells. ATP concentrations were therefore measured in cells that were supposed to contain various concentrations of intracellular FFA. It was observed that adrenaline, or agents such as caffeine, brought about a marked decrease in ATP concentrations. On the other hand the decreased ATP concentrations observed in adrenaline-treated

cells were restored towards normal by the superimposed addition of insulin. The adrenaline effect could be mimicked by increasing FFA concentration in the medium.

Possible relationships between lipolysis, FFA concentrations, electrolytes, glucose uptake and energy availability will be discussed (in collaboration with M. Touabi, I. Bihler and J. D. Vassalli).

Transport of Lipid across the Small Intestine

By G. HÜBSCHER. (*Department of Biochemistry, The Medical School, University of Nottingham*)

During the past 10 years a great deal of information has emerged about the mechanism of absorption of fatty acids and glycerides in the small intestine. Most of this information is well documented (see Senior, 1964; Johnston, 1968). The emphasis of this presentation will therefore be on current research in outstanding problems of fat absorption.

One of these is the mechanism of entry of long-chain fatty acids and monoglycerides into the columnar epithelium of the small intestine. After partial hydrolysis of dietary glycerides in the lumen of the small intestine, the liberated long-chain fatty acids and 2-monoglycerides form, with conjugated bile salts, anionic micelles (see Hofmann, 1968). It is in this form that the lipids come into contact with the brush-border membrane.

Although it is generally accepted that micellar lipid is better absorbed than a lipid emulsion, micelles are not an obligatory carrier of lipids across the brush-border membrane. Thus monomolecular solutions of palmitate and oleate are efficiently absorbed (Simmonds, Redgrave & Willix, 1968). This is in agreement with the observation that lipids and bile salts are absorbed individually and not as multiple-component micelles (Thornton, Vahouny & Treadwell, 1968). At variance with these results is the finding that rings of hamster small intestine take up taurodeoxycholate and oleate in approximately equimolar amounts (Gordon & Kern, 1968). Moreover, on the basis of experiments with everted sacs of rat small intestine, Small & Dietschy (1968) have suggested that intact micelles may cross the brush-border membrane. If this were so, bile salts would have to be excreted back into the intestinal lumen because bile salts are absorbed in the lower part (Playoust & Isselbacher, 1964) and fatty acids plus monoglycerides in the upper part (Borgström, Lundh & Hofmann, 1963) of the small intestine.

Theoretical considerations based on the vapour pressure of the solute (Coe, 1966) or on the activation energy required for the passage of lipid from an aqueous to a lipid phase (Danielli, 1964) do not exclude passive transport of lipid across cell membranes (see also Hogben, 1966). Lipid transport is also indicated to be energy-independent

from various experimental studies (Johnston & Borgström, 1964; Strauss, 1966), although the entry is reported to be Na^+ -dependent and to show characteristics typical for a saturation phenomenon (Lyon, 1968). By analogy with the erythrocyte membrane, there is experimental evidence for an energy-dependent entry of palmitate into cell membranes (Shohet, Nathan & Karnovsky, 1968).

The uptake of lipid by the columnar epithelium is of importance as it is the rate-limiting step as compared with the subsequent intracellular biosynthesis of triglycerides (Raghavan & Ganguly, 1967; Hamilton, Webb & Dawson, 1969).

A further topic of current interest is the biosynthesis of triglycerides in the mucosa of the small intestine. The two biosynthetic routes (the glycerophosphate pathway and the monoglyceride pathway; Clark & Hübscher, 1960) have been found by several investigators to occur in the intestinal mucosa of a number of species of laboratory animals. More recently, these pathways have been described in intestinal preparations of chicken, pigs (Bickerstaffe & Annison, 1969) and sheep (Leat & Cunningham, 1968; Bickerstaffe & Annison, 1969).

The monoglyceride pathway is, under normal dietary conditions, the major route of glyceride biosynthesis in rats (Mattson & Volpenhein, 1964; Paris & Clément, 1968) and probably also in man (Kayden, Senior & Mattson, 1967), but only of minor importance in sheep (Bickerstaffe & Annison, 1969). The enzymes catalysing the biosynthesis of triglycerides via the monoglyceride pathway have been purified 70–100-fold from hamster small intestine (Rao & Johnston, 1966). The purified synthetase contained acyl-CoA synthetase, acyl-CoA-monoglyceride acyltransferase and acyl-CoA-diglyceride acyltransferase, which were enriched to approximately the same degree. The purified synthetase, as well as the original microsomal fraction, shows a requirement for enzyme-bound CoA (Rao & Johnston, 1967). Preincubation of the synthetase with palmitoyl-CoA or with ATP, CoA and Mg^{2+} results in the formation of enzyme-bound CoA (or moiety of CoA) that, in a subsequent incubation, can be demonstrated by a CoA-independent biosynthesis of triglyceride from palmitate, monoglyceride and ATP.

Enzyme-bound CoA is also involved in the biosynthesis of triglycerides via the glycerophosphate pathway (Rao & Johnston, 1967). Two further aspects of this pathway should be mentioned. One is related to the role of dihydroxyacetone phosphate as an alternative acyl acceptor, so far described only in subcellular fractions from mammalian liver (Hajra, 1968; Hajra & Agranoff, 1968). The other deals with the requirement for a specific phosphatidate phosphohydrolase, which acts on membrane-bound biosynthetically produced phos-