blood glycerides was ten times higher in livers from fed rats (0.69% of the dose/min) compared with livers from rats starved for 24h (0.065%). Production of ^{14}C -labelled ketone bodies in the steady state was presumably a result of lipolysis of liver ^{14}C -labelled glycerides. It was higher in the livers of starved rats ($0.0278 \pm 0.008\%$ of the dose/min) compared with those of fed rats ($0.0013 \pm 0.0002\%$ of the dose/min). Production of total ketone bodies was also higher in livers from starved rats. Addition of glucagon to perfused livers from fed rats depressed the rate of formation of ^{14}C -labelled very low density lipoproteins and increased the rate of formation of ^{14}C -labelled ketone bodies; it was without effect on livers from starved rats. Addition of insulin to livers from starved rats resulted in an increased output of ^{14}C -labelled very low density lipoproteins and a reduction in formation of ^{14}C -labelled ketone bodies.

The support of the Wellcome Trust is gratefully acknowledged.

Bewsher, P. D. & Ashmore, J. (1966). *Biochem. biophys. Res. Commun.* **24**, 431.

Mayes, P. A. & Felts, J. M. (1966). *Proc. Eur. Soc. Study of Drug Toxicity*, vol. 7, p. 16; *Excerpta med. Congr. Ser.* no. 115.

Williamson, J. R., Herczeg, B., Coles, H. & Danish, R. (1966). *Biochem. biophys. Res. Commun.* **24**, 437.

Direct Stimulation by Insulin and Fructose of Very-Low-Density Lipoprotein Secretion by the Perfused Liver

By D. L. TOPPING and P. A. MAYES. (*Division of Biochemistry, Royal Veterinary College, London N.W.1, U.K.*)

Perfused livers from fed male rats (340–360g) were infused with [$1\text{-}^{14}\text{C}$]oleate, to maintain a concentration of $0.9\text{ }\mu\text{mol/ml}$ of serum. A trace quantity of [$1\text{-}^3\text{H}$]acetate was also infused continuously. In some experiments fructose was infused to maintain a concentration of $25\text{--}30\text{ mg/100ml}$ of blood. Where appropriate, bovine insulin was added to maintain a minimum concentration of 56 ng/ml . High physiological concentrations of rat insulin were obtained in some experiments by pre-treating the blood-donor rats with intraperitoneally administered glucose. The perfusions were initiated with defibrinated rat blood that had been dialysed against Krebs–Henseleit bicarbonate buffer. An equal volume of similar but undialysed blood was added subsequently. The blood glucose concentration was stabilized at $200\text{--}300\text{ mg/100ml}$. Other experimental details were as described by Mayes & Felts (1966).

In control perfusions, production of very-low-density lipoprotein (density <1.006) averaged $27\text{ }\mu\text{mol}$ of triglyceride fatty acids/h per liver. In the presence of higher concentrations of rat insulin or fructose the average rate of secretion rose to $39\text{ }\mu\text{mol/h}$ per liver. However, when bovine insulin or an increased concentration of rat insulin was present in addition to fructose there was a marked augmentation of the rate of secretion to 56 and $53\text{ }\mu\text{mol/h}$ per liver respectively. Incorporation of ^{14}C -labelled long-chain free fatty acids into very-low-density lipoprotein showed similar trends. There was, however, a stimulation of incorporation in the presence of higher concentrations of rat insulin. Oxidation of ^{14}C -labelled free fatty acids to $^{14}\text{CO}_2$ was decreased by insulin and fructose added separately and was decreased further when both were present together. Lipogenesis, as indicated by the incorporation of [^3H]acetate into triglyceride of very-low-density lipoprotein, was stimulated by the presence of fructose and insulin and was inversely related to the oxidation of ^{14}C -labelled free fatty acids.

The following hypothesis is suggested to explain the combined effect of insulin and fructose in stimulating very-low-density lipoprotein secretion. The decreased oxidation of ^{14}C -labelled free fatty acids may indicate that insulin enhances net esterification of free fatty acids (acyl-CoA), thus removing inhibition of lipogenesis by acyl-CoA. The pathway would then be open for rapid lipogenesis from fructose, followed by enhanced secretion of very-low-density lipoprotein. Present results may help to explain the correlation between atherosclerosis, sucrose intake and the concentration of serum insulin (Yudkin, Szanto & Kakkar, 1969).

The support of the Medical Research Council is gratefully acknowledged. Crystalline bovine insulin was generously given by Burroughs Wellcome and Co.

Mayes, P. A. & Felts, J. M. (1966). *Proc. Eur. Soc. Study of Drug Toxicity*, vol. 7, p. 16; *Excerpta med. Congr. Ser.* no. 115.

Yudkin, J., Szanto, S. & Kakkar, V. V. (1969). *Postgrad. med. J.* **45**, 608.

The Site of Fatty Acid Biosynthesis in the Avocado Pear

By P. J. WEAIRE and R. G. O. KEKWICK. (*Department of Biochemistry, University of Birmingham, Birmingham 15, U.K.*)

Barron, Squire & Stumpf (1961) have described the incorporation of [$1\text{-}^{14}\text{C}$]acetate into fatty acids by the mitochondrial fraction from homogenates of the mesocarp of the avocado pear. Subsequently Yang & Stumpf (1965) reported the incorporation

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^{14}C]acetate into fatty acids.

Although the supernatant did not incorporate [1^{14}C]acetate or [2^{14}C]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^{14}C]acetate.

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

Baker, J. E., Elvin, L. G., Biale, J. B. & Honda, S. I. (1968). *Pl. Physiol., Lancaster*, **43**, 2001.

Barron, E. J., Squire, C. & Stumpf, P. K. (1961). *J. biol. Chem.* **236**, 2610.

Hobson, G. E. C., Lance, C., Young, R. E. & Biale, J. B. (1966). *Nature, Lond.*, **209**, 1242.

Yang, S. P. & Stumpf, P. K. (1965). *Biochim. Biophys. Acta*, **98**, 19.

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

By D. REICHL (introduced by L. G. GOODWIN).
(Department of Biochemistry, Nuffield Institute of Comparative Medicine, The Zoological Society of London, Regent's Park, London N.W.1, U.K.)

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 $\mu\text{g}/\text{rat}$), 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