Since cholesterol is synthesized in many tissues but is removed from the body almost entirely via the liver, there must also be a net transfer of cholesterol from the tissues to the plasma. It is possible that only free cholesterol can be transferred in this way, since cholesteryl esters accumulate in various organs in the rare condition in which there is a deficiency of lysosomal cholesteryl ester esterase.

The plasma cholesteryl esters turn over as a result of continual hydrolysis and resynthesis. In man the fractional rate of turnover of the total esterified cholesterol in the plasma is such that about 100 mg is renewed/h. In human plasma all the cholesteryl esters (irrespective of their fatty acid moiety) turn over at the same fractional rate, but the cholesteryl esters in the high-density lipoprotein turn over more rapidly than do the low-density-lipoprotein cholesteryl esters. In man the plasma cholesteryl esters do not originate in the liver but are formed in the plasma by the action of lecithin (phosphatidylcholine)-cholesterol acyltransferase, which transfers the fatty acyl residue on C-2 of the plasma lecithin to free cholesterol in the high-density lipoproteins. The cholesteryl esters so formed are then transferred to the low-density and very-low-density lipoproteins, possibly by exchanging with triglyceride. In the familial disease in which the acyltransferase is absent from the plasma no cholesteryl esters are present in the plasma in the fasting state. As expected, in human plasma the composition of the fatty acids in the cholesteryl esters is similar to that of the fatty acids on C-2 of lecithin. In rats the cholesteryl esters of the high-density lipoproteins are formed by the acyltransferase reaction, but the cholesteryl esters of the very-low-density lipoproteins (which have a fatty acid pattern different from that of the C-2 of lecithin) are probably formed in the liver and small intestine.

The turnover of the plasma cholesterol is influenced by dietary, genetic and other factors. A diet rich in polyunsaturated fats lowers the concentration of the plasma cholesterol, possibly by promoting its catabolism and excretion. Cholestyramine lowers the plasma cholesterol concentration by stimulating the formation of bile acids. This results in increased hepatic synthesis of cholesterol. There is thus an increase in both the fractional and the absolute rate of turnover of the plasma cholesterol. Other drugs alter the turnover of the plasma cholesterol by interfering with the intestinal absorption of cholesterol, by inhibiting its endogenous synthesis, by stimulating the excretion of the plasma cholesterol, or by a combination of these effects. The raised plasma cholesterol concentration in familial hyper- β -lipoproteinaemia is associated with a diminished fractional rate of turnover of the plasma cholesterol, as shown by the presence of a subnormal faecal

clearance rate (ml of plasma cleared of cholesterol via the faeces/day), but a normal absolute rate of turnover. In this disease there is also a subnormal fractional rate of turnover of the apoprotein of the low-density lipoproteins. These findings are consistent with defective removal of low-density lipoproteins, but other interpretations are equally plausible.

The Mechanism of Removal of Lipoprotein Triglyceride from the Plasma

By D. S. ROBINSON,* (*Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU, U.K.*)

The major physiological importance of the plasma lipoproteins derives from their function as carriers of triglyceride, most of which is present in the plasma in the chylomicrons and very-low-density lipoproteins, derived respectively from the intestine and the liver. The turnover time of the plasma chylomicron and very-low-density-lipoprotein triglyceride is much shorter than that of other lipoprotein lipid: its half-life in the rat, for example, is only a few minutes. Moreover, although the rate of influx of triglyceride into the plasma may increase markedly in particular physiological circumstances (for instance, during the absorption of dietary fat), the triglyceride concentration in the plasma normally increases only moderately: that is, the rate of removal rises to balance the increased rate of entry.

It can be concluded that a mechanism for the rapid removal of triglyceride from the plasma exists and that this is not saturated under normal conditions. It is now established that this removal mechanism involves hydrolysis of the chylomicron and very-low-density-lipoprotein triglyceride by the enzyme clearing-factor lipase (lipoprotein lipase). It appears that this hydrolysis occurs at or close to the luminal surface of the capillary endothelial cells and that the free fatty acids released then pass across the endothelial cells into the tissues. Particular lipoprotein peptides in the chylomicrons and very-low-density lipoproteins may well play a specific role in the association of these complexes with the enzyme at the endothelial cell surface. Though most of the triglyceride fatty acids removed from the blood are taken up by the extrahepatic tissues by the action of clearing-factor lipase, the possibility of some uptake by the liver by a different process cannot be excluded.

Changes in the extent of uptake of plasma triglyceride fatty acids by particular tissues occur when the physiological state changes: for example, starvation results in a fall in uptake by adipose

* Present address: Department of Biochemistry, University of Leeds, Leeds LS2 9LS, U.K.

tissue and an increase in uptake by muscle. These alterations in the site of removal can be correlated with corresponding changes in the clearing-factor lipase activity of the tissues concerned, and the enzyme therefore seems to play a directive role in determining the pattern of uptake of triglyceride fatty acids.

The changes in activity of clearing-factor lipase that occur in adipose tissue *in vivo* can be simulated *in vitro*. Thus high levels of enzyme activity can be induced by incubating epididymal fat-bodies from starved rats in appropriate media *in vitro* and similar results can be obtained with isolated fat-cells. Studies with such systems suggest that: (a) the induction process is under hormonal control,

which may be mediated through adenosine cyclic 3':5'-monophosphate, either directly or through secondarily induced changes in free fatty acid or ATP concentrations in the fat-cell; (b) enzyme in the fat-cell may be the precursor of the functional enzyme at the capillary endothelial cell surface.

Clinical Disorders of the Plasma Lipoproteins

By BASIL M. RIFKIND. (*Clinic for Lipoprotein Disorders, Royal Infirmary, Glasgow C.4, U.K.*)

Significant advances continue to be registered in the fields of hyperlipoproteinaemia and hypolipoproteinaemia. Selected aspects of these advances will be reviewed.

COMMUNICATIONS

Phenol Oxidase Activity in the Larva of the Blowfly *Calliphora erythrocephala*

By G. M. PRICE and L. HUGHES. (*Agricultural Research Council Unit of Invertebrate Chemistry and Physiology, University of Sussex, Brighton BN1 9QJ, U.K.*)

The presence of a pupation-inhibiting factor in the blowfly larva has been demonstrated (Price, 1970) and it was suggested that this factor might be acting as a phenol oxidase inhibitor. To determine the site at which the inhibitor was produced, various tissues isolated from blowfly larvae were incubated for 10 min with 0.1 ml of haemolymph (phenol oxidase source) in 2.7 ml of 0.1 M-phosphate buffer, pH 6.7. Methylcatechol (final concn. 1.3 mM) was added and incubation was continued for 30 min at 30°C, after which the extinction of the solution was measured at 475 nm. It was found that the salivary glands (10) and guts (4) inhibited the phenol oxidase activity, whereas brains (10), fat-body (1) and Malpighian tubules (4) had little effect and muscle (half) and cuticle (1) activated the enzyme. The observation that the salivary glands produce an inhibitor confirms that made by Thomson & Sin (1970), and the production of an activator by the cuticle has been observed by a number of workers, notably Sekeris & Mergenhagen (1964). After salivary glands and cuticles were incubated separately in phosphate buffer, pH 6.7, at 2°C overnight and then removed, inhibitor and activator respectively were found in the medium. When salivary glands were suspended by a hair so that the duct was clear of buffer and only the gland was immersed, inhibitor was subsequently found in the buffer, showing that inhibitor was released through the walls of the gland. When solutions containing the inhibitor and activator were mixed an antagonistic effect was observed.

On elution from a column of Sephadex G-25 the inhibitor and activator both behaved as proteins. After disc electrophoresis of the inhibitor solution on polyacrylamide gel staining with Amido Black showed the presence of two protein bands, whereas with the activator solution at least ten bands were obtained.

A technique was developed by which the larval cuticle could be split into three layers, outer, middle and inner. Activator was found in the outer and inner layers but was absent from the middle layer. When the inner layer was scraped on the surface to which the epidermal cells would be attached *in vivo*, it produced much less activator than did an unscraped layer. The results indicate that much of the activator of this layer probably originates in the epidermal cells attached to it, the other main source of activator being in the outer layer, which is made up wholly or partly by the epicuticle.

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Enzyme Insolubilization with Cross-Linked Polyacryloylaminoacetaldehyde Dimethyl-acetal

By R. EPTON, J. V. McLAREN and T. H. THOMAS. (*Department of Physical Sciences, The Polytechnic, Wolverhampton WV1 1LY, U.K.*)

Water-insolubilization of enzymes by cross-linking with monomeric dialdehydes is well documented (Quiocho & Richards, 1964; Ogata, Othensen & Svendsen, 1968; Avrameas & Ternynck, 1969), but enzyme coupling with insoluble polyaldehydes has received little attention. Dispersion