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

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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

over a preformed polymer surface is conducive to both enzyme economy and micro-environmental control. We now report the insolubilization of trypsin, papain, α -amylase and dextranase with polyaldehydes derived from cross-linked polyacryloylaminoacetaldehyde dimethylacetal (Enzacryl Polyacetal), a new commercial matrix for enzyme binding.

By controlled acid hydrolysis of the polyacetal, polyaldehydes of various water regains were prepared. Although maximum aldehyde content (5.0 mequiv./g of polymer) was consistent with maximum water regain (2.2 ml/g of polymer), aldehyde content and swelling were not simply related.

Water-insoluble trypsin derivatives were prepared by stirring a solution of the enzyme in 25 mM-phosphate buffer, pH 7.5, with polymers containing 2.2–5.0 mequiv./g of aldehyde. The resulting conjugates were washed exhaustively with 25 mM-phosphate buffer and buffered 1% casein solution. The amount of enzyme bound (10–50 mg/g of polymer) depended more on water regain than on aldehyde content. Tryptic activities towards N^α -benzoylarginine ethyl ester were 16–25% of those of the free enzyme. Insoluble papain derivatives were similarly prepared.

No significant activity loss towards N^α -benzoylarginine ethyl ester occurred with insolubilized preparations of either enzyme on repeated re-use. Further, resolubilized activity could not be detected in N^α -benzoylarginine ethyl ester digests after removal of the insolubilized enzyme. However, although the insolubilized enzymes could be used repeatedly to digest casein, with excellent recovery of activity, some slight resolubilization of enzyme was always observed, possibly as a result of the potentially reversible azomethine binding. Affinity for the macromolecular substrate is probably sufficient to cause significant partitioning of insolubilized enzyme between carrier and aqueous phase.

Similar results were observed with polyaldehyde-bound α -amylase and dextranase acting on starch and dextran respectively. Very slow enzyme release with simultaneous regeneration of aldehyde groups could be of importance in industrial reactors, since the activity of the insoluble catalyst could be boosted periodically with fresh enzyme.

Hydrophilic character of the derivatives is conferred by acetal, hemiacetal and aldehydrol groups. This provides a new micro-environment for enzyme insolubilization that has the potential for modification subsequent to enzyme coupling.

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Heterotropic Co-operative Effects between Amino Acid Substrates of Phenylalanine Hydroxylase

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Coulson, Wardle & Jepson (1967) reported the observation of a homotropic co-operative effect of both phenylalanine and 4-fluorophenylalanine when used as substrates for phenylalanine hydroxylase, and speculated as to whether this enzyme was an allosteric protein. Kaufman & Fisher (1970) have reported the existence of a subunit structure for the purified enzyme that reinforces this possibility.

Using ^{14}C -labelled substrates with a partially purified enzyme preparation we have demonstrated that the three previously reported amino acid substrates for phenylalanine hydroxylase, namely phenylalanine, 4-fluorophenylalanine and tryptophan, as well as showing expected competitive effects at high substrate concentrations, also show heterotropic co-operative effects at low substrate concentrations.

Whereas homotropic co-operative effects in a multi-substrate reaction can be accounted for by a kinetic model in which the substrates can bind in either order to form the same ternary complex (Ferdinand, 1966), a heterotropic co-operative effect will not fit this model. The results can be accounted for by either an allosteric model (Monod, Wyman & Changeux, 1965) or a ligand-exclusion model (Fisher, Gates & Cross, 1970), but the final distinction will depend on whether conformational changes in the protein can be shown to accompany substrate binding.

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