retinol is probably by transesterification with endogenous lipids such as phospholipids, whereas the monoglycerides and lysophosphatides are esterified directly through acyl-CoA. In contrast with this general pattern of absorption of various lipids retinoic acid follows a completely different mechanism, in that it is rapidly absorbed through the portal route and is quickly excreted through the bile (Fidge *et al.*, 1968).

David, J. S. K. & Ganguly, J. (1967) *Indian J. Biochem.* **4**, 14

Fidge, N. H., Shiratori, T., Ganguly, J. & Goodman, DeW. S. (1968) *J. Lipid Res.* **9**, 103

Raghavan, S. S. & Ganguly, J. (1969) *Biochem. J.* **113**, 81

Subbaiah, P. V. & Ganguly, J. (1970) *Biochem. J.* **118**, 233

Subbaiah, P. V., Sastry, P. S. & Ganguly, J. (1970) *Biochem. J.* **118**, 241

Structure and Enzymic Properties of Phospholipase A from Porcine Pancreas

By G. H. DE HAAS (*Biochemistry Laboratory, State University, Utrecht, The Netherlands*)

Phospholipase A (EC 3.1.1.4) is an ubiquitously occurring enzyme that catalyses the hydrolysis of 1,2-diacyl-*sn*-glycerol-3-phosphatides into 1-mono-acyl-*sn*-glycerol-3-phosphatides (lysophosphatides) and free fatty acids. In mammalian pancreas this enzyme occurs as an inactive zymogen that, after its transport to the intestine, is converted by limited tryptic proteolysis into the active enzyme. The zymogen, as well as the active enzyme, was isolated from porcine pancreas. Both proteins are low-molecular-weight compounds (mol.wt. about 14 000), each consisting of one single polypeptide chain intramolecularly cross-linked by six disulphide bridges. The amino acid sequence and the positions of the disulphide bonds have been determined and the mechanism of the conversion of the precursor into the active enzyme has been elucidated.

By using a series of different synthetic phospholipids and intermediates, in which the polar head groups as well as the apolar acyl chains were modified, the minimal substrate requirements of the enzyme were determined. The enzyme has an absolute requirement for Ca^{2+} , and a mechanism based on kinetic analysis will be proposed. The kinetic parameters V_{max} and K_m were determined, and their dependence on pH and ionic strength will be discussed in relation to the amino acid side chains involved in binding and catalysis. The synthesis and kinetic behaviour of active-site-protecting competitive inhibitors will be reported, as well as their use in chemical modification studies of active-centre residues. The pronounced preference of the enzyme to attach only substrates that form organized lipid/

water interfaces will be demonstrated, and the implications involved in kinetic analysis will be discussed.

The Role of Clearing-Factor Lipase in the Removal of Triglyceride Fatty Acids from the Plasma

By D. S. ROBINSON (*Department of Biochemistry, University of Leeds, Leeds LS2 9LS, U.K.*)

Removal from the blood of the triglyceride fatty acids of chylomicrons and very-low-density lipoproteins occurs by virtue of the hydrolysis of the triglycerides in the capillary beds of the extrahepatic tissues by the enzyme clearing-factor lipase (lipoprotein lipase).

The extent to which the plasma triglyceride fatty acids are taken up by a particular tissue depends on the clearing-factor lipase activity of that tissue, relative to its activity in other tissues. Moreover, activity of the enzyme at a particular tissue site can change rapidly. Hence the enzyme has a directive role in the distribution of the plasma triglycerides. Evidence for this role is provided by the reciprocal changes in muscle and adipose-tissue clearing-factor lipase activity that occur with changes in nutritional state and that are associated with reciprocal changes in the extent of triglyceride fatty acid uptake by these tissues, and by the finding that the activity of clearing-factor lipase in the mammary gland is high only when uptake of triglyceride fatty acid occurs during lactation.

Alterations in the clearing-factor lipase activity of particular tissues may involve both changes in the amount of enzyme present and the conversion of one form of the enzyme into another. Evidence for the existence of more than one form of the enzyme in adipose tissue is available. There is, moreover, evidence to suggest that the changes in activity of the enzyme are under hormonal control and that such hormonal control is exerted, directly or indirectly, through changes in the tissue content of adenosine 3':5'-cyclic monophosphate.

Synthesis of Naturally Occurring Phosphonolipids

By ERICH BAER (*Banting and Best Department of Medical Research, University of Toronto, Toronto 5, Ont., Canada*)

The synthesis of phosphonic acid analogues of ester and ether phosphatidylethanolamines, phosphatidylserines and phosphatidylcholines (ester and ether kephalins and ester and ether lecithins) and

their acyl-free moieties, as well as two sphingolipids, is reviewed.

Wax Esters: Major Marine Metabolic Energy Sources

By A. A. BENSON and RICHARD F. LEE (*Scripps Institution of Oceanography, University of California, San Diego, La Jolla, Calif. 92037, U.S.A.*)

The importance of polyunsaturated wax esters as metabolic reserves in marine copepods has been recognized by Lee *et al.* (1970). These major zooplankton of the world's oceans convert the highly unsaturated triglycerides of diatom and dinoflagellate algae on which they graze into wax esters comprised of polyunsaturated fatty alcohols and polyunsaturated fatty acids. Metabolic-turnover experiments have revealed that the animals first utilized the triglyceride components of their stored lipids and then more slowly metabolized their wax ester reserves. Although all copepods store wax ester, those of temperate and polar waters have adapted to their environment and long unproductive winters by accumulating up to 75% of their body weight as wax ester. In warm tropical waters the continuity of the food supply makes long-term energy supplies unnecessary and wax ester storage is minimal.

Wax ester accumulation and metabolism in marine animals appears to be controlled through wax lipase. Stage V copepodites, immediate precursors of the egg-bearing adults, must store energy reserves for winter 'hibernation', moulting, mating and egg production. At this stage they lack wax lipase activity, although triglyceride lipase is relatively active. Stage V copepodites of *Calanus plumchrus* contain up to 60% lipid, which is comprised of 90% wax ester. After starvation for 50 days their triglyceride content diminished to zero. *Gaussia princeps* lipid composition changed from 49% wax ester and 18% triglyceride to 61% wax ester and 2% triglyceride during 5 days' starvation (Lee *et al.*, 1972).

Anchovies, herring, sardines and salmon, all predators on wax-rich animals, possess active wax lipases that cleave the wax esters quickly, leaving no trace of wax ester in serum lipoproteins. Wax digestion in fishes occurs in the mucosa of the pyloric caecum. Wax globules are adsorbed in the folds of mucosal surfaces. Blood serum of young salmon feeding on a high-wax diet is rich in triglyceride and cholesteryl ester and contains no wax ester. The site of fatty alcohol oxidation lies in the intestinal mucosa. A mechanism for conversion into fatty acyl ester involving membrane-bound ether and plasmalogen intermediates appears probable.

The copepod wax esters are among the most un-

saturated lipids in Nature, being derived from algal triglycerides containing $C_{22:6}$, $C_{20:5}$ and $C_{20:6}$ fatty acids. The fatty alcohol moiety is relatively unsaturated, 13% $C_{16:0}$, 18% $C_{20:1}$, 41% $C_{20:4}$ and 8% $C_{22:6}$ alcohol in *Calanus helgolandicus* (Lee *et al.*, 1971). Carnivorous deep-sea copepods contain up to 50% of $C_{16:0}$ alcohol, reflecting their dietary differences. The fatty acids of the wax esters of near-surface herbivorous copepods are less saturated than the alcohol moieties. They included 23% $C_{20:5}$ and 14% $C_{20:4}$ fatty acids in *Calanus hyperboreus*. It is observed that surface-feeding copepod species in temperate and polar waters store their highly unsaturated wax ester in a very large abdominal oil sac, whereas the bathypelagic animals of the deep sea, living at temperatures near 0°C, store relatively saturated wax esters. This is contrary to the usual variation in unsaturation with depth. These animals store their lipid in dispersed globules throughout their tissues. It is presumed that this is an evolutionary adaptation to facilitate utilization of the more saturated wax esters at the lower temperatures.

Wax esters and triglycerides exhibit different densities and compressibilities relative to that of sea water. We have isolated both in large quantities for measurement of their physical properties. The important vertical-migrating animals of the sea have selected wax ester for buoyancy regulation. All animals living below 700m have been observed to contain wax ester (Lee *et al.*, 1972). The most numerous fishes of the seas, the bristlemouth (cyclothone) fishes, Gonostomatidae and the lantern fish, Myctophidae, contain 53% wax ester in their lipids; the coelacanth contains 70% wax ester (Nevenzel, 1970). All members of bathypelagic food chains contained large amounts of lipid rich in wax ester. The utilization of lipid in the marine environment, like that in desert animals without available fresh water, allows extensive production of metabolic water. By selection of wax esters in addition to triglyceride, certain marine animals are able to exert added control over their lipid metabolism and to migrate vertically with less expenditure of energy. Wax ester accumulation appears to have been an important evolutionary adaptation.

This work was supported by the National Science Foundation (U.S.) and the Foundation for Ocean Research. Technical assistance of Mrs. E. Baker and helpful discussions and collaboration with Dr. V. Swanson, Dr. D. J. Faulkner, Dr. J. Hirota and Dr. J. C. Nevenzel are gratefully acknowledged.

Lee, R. F., Nevenzel, J. C. & Paffenhöfer, G.-A. (1970) *Science* **167**, 1508

Lee, R. F., Nevenzel, J. C. & Paffenhöfer, G.-A. (1971) *Mar. Biol.* **9**, 99

Lee, R. F., Hirota, J. & Barnett, A. M. (1972) *Deep Sea Res.* in the press

Nevenzel, J. C. (1970) *Lipids* **5**, 308