- Sun, G. Y. & Horrocks, L. A. (1971) *J. Neurochem.* **18**, 1963
 Webster, G. R. & Cooper, M. (1968) *J. Neurochem.* **15**, 795
 Wells, M. A. & Dittmer, J. C. (1967) *Biochemistry* **6**, 3169

Cerebroside Sulphate Biosynthesis in Brain

By B. K. BACHHAWAT, SHALINI KOKRADY and S. SAXENA (*Neurochemistry Laboratory, Department of Neurological Sciences, Christian Medical College Hospital, Vellore, S. India*)

Earlier reports from our laboratory have shown the presence in brain of a sulphotransferase that catalyses the transfer of sulphate from adenosine 3'-phosphate 5-sulphatophosphate to an endogenous protein-bound galactose cerebroside. This enzyme has been partially purified from sheep brain, and was found to be activated by EDTA and thiols such as GSH and cysteine. Attempts to use exogenous cerebroside as a substrate for this enzyme were unsuccessful, and the use of detergents such as sodium deoxycholate, Triton X-100 and Brij-96 resulted in a marked decrease in its activity (Bhandrai & Bachhawat, 1967). Recently it has been possible to purify the endogenous acceptor partially by DEAE-Sephadex chromatography, and indirect evidence has been obtained that indicates that it is a protein-bound cerebroside. This sulphotransferase system seems to be distinct from the one described by Cumar *et al.* (1968) and McKhann & Ho (1967). Recently, by using purified microsomal and synaptosomal fractions, we have been able to show that these preparations are very active in our system and that detergents markedly inhibit the activity. Solubilization of the microsomal fraction by deoxycholate led to a marked decrease in the activity, even though detectable amounts of cerebroside were present in the solubilized preparation, which further supports our previous observation that this cerebroside sulphotransferase is distinct. It also explains the failure of Kopaczky & Radin (1965) to detect the formation of any sulphatide after injections into rats of cerebroside containing radioactive non-hydroxy fatty acid. This can be explained from our observation, because exogenous cerebroside is not an appropriate substrate for the synthesis of the product in our system. T.l.c. patterns of the isolated cerebroside suggest that both hydroxy and non-hydroxy fatty acids containing sulphatides are synthesized in our system.

- Bhandari, V. R. & Bachhawat, B. K. (1967) *Proc. 1st. Int. Meet. International Society for Neurochemistry, Strasburg*, p. 24
 Cumar, F. A., Barra, H. S., Maccioni, H. J. & Caputto, R. (1968) *J. Biol. Chem.* **243**, 3807
 Kopaczky, K. C. & Radin, N. S. (1965) *J. Lipid Res.* **6**, 140
 McKhann, G. M. & Ho, W. (1967) *J. Neurochem.* **14**, 717

Absorption of Lipids

By J. GANGULY, P. V. SUBBAIAH and S. PARTHASARATHY (*Department of Biochemistry, Indian Institute of Science, Bangalore-12, India*)

Recent work on the absorption of triglycerides has shown that during the digestive phase the dietary triglycerides are hydrolysed by the pancreatic lipase mainly to the 2-monoglyceride stage, after which the products of lipolysis appear in a micellar solution along with bile salts. From such a micellar solution the 2-monoglycerides and free fatty acids diffuse into the mucosal cell to be reassembled into triglycerides. By using triglycerides containing ^{14}C -labelled fatty acids at different positions Raghavan & Ganguly (1967) have produced convincing evidence that during the process of digestion and resynthesis about 80% of the fatty acid at the β -position of the triglycerides is retained at the same place.

Our sustained work on the mechanism of absorption of retinyl and cholesteryl esters has similarly shown that the retinyl esters are partially hydrolysed while in the fat phase, after which the retinol and its esters appear in a micellar solution (David & Ganguly, 1967). Both retinol and its esters then diffuse into the cell, where the hydrolysis of the ester is completed and retinol is re-esterified preferentially with palmitic acid. The cholesteryl esters, on the other hand, are totally hydrolysed while in the fat phase, so that only the de-esterified sterol appears in the micelle; the micellar sterol is then admitted into the cell, where it is re-esterified.

We have also demonstrated that the rat mucosal cell is rich in phospholipase A activity and that considerable proportions of this activity are localized in the brush border (Subbaiah & Ganguly, 1970). Our work further demonstrated the presence of a very active acyl-CoA-lysophosphatidylcholine acyltransferase in the microsomal fraction of rat mucosa (Subbaiah *et al.*, 1970). Sufficient evidence has since been gathered to demonstrate that during absorption the fatty acid at the β -position of phosphatidylcholine (lecithin) undergoes rapid turnover. Here also it appears that the phosphatidylcholine is hydrolysed by the pancreatic phospholipase A to lysophosphatidylcholine, which being readily soluble in water can diffuse into the cell to be reacylated by the mucosal acyltransferase.

Thus it appears that there are certain similarities in the absorption of different lipids, in that they are hydrolysed by the lipolytic enzymes in the intestinal lumen to such a state that they can be made water-soluble and thereby can readily diffuse into the mucosal cell, to be re-esterified intracellularly to the parent compounds and transported through the lymph. The re-esterification of cholesterol and

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