with such storage diseases occurs at a normal rate. The second is the development of techniques for introducing radiotracer elements into the various lipid molecules. Investigations with labelled materials provided for the identification of the nature of the enzymic defects in Gaucher's disease, metachromatic leucodystrophy, Niemann-Pick disease and Fabry's disease in the short period of time from 1965 to 1967. These findings established the still unbroken precedent that all of the lipidoses are due to attenuation of specific catabolic enzymes normally required for the metabolic disposal of the accumulating lipid. There are now nine known diseases of this nature, and the respective enzymic abnormalities are indicated in Table 1. This hard-won knowledge has recently been put to practical clinical use. We can now accurately diagnose homozygous (afflicted) individuals, we can identify heterozygous carriers of these recessive diseases and, most importantly, procedures have now been developed for the accurate prenatal monitoring of pregnancies at risk for all of the lipid-storage diseases. The second portion of the presentation will deal with the profound alterations of ganglioside pattern and synthesis in DNA- and RNA-tumorigenic-virus-transformed cell lines.

Two Cerebral Sphingolipidoses (Metachromatic Leucodystrophy and Infantile Amaurotic Idiocy) as Human Inborn Errors of Metabolism

By H. JATZKEWITZ (*Max-Planck-Institut für Psychiatrie, Neurochemische Abteilung, München, West Germany*)

Both metachromatic leucodystrophy and infantile amaurotic idiocy arise from an accumulation of sphingoglycolipids. In cases of metachromatic leucodystrophy cerebroside sulphates (i.e. sulphatides or ceramide-galactose sulphate) accumulate. In patients suffering from infantile amaurotic idiocy gangliosides (i.e. ceramide-oligosaccharides + *N*-acetylneuraminic acid residues) and to a smaller extent their neuraminic acid-free residues are stored. This takes place especially in the brain, but also in other tissues. The major monosialoganglioside in the brain contains a single *N*-acetylneuraminic acid (sialic acid) residue, derived from a tetrahexosylceramide, and has the following structure:

where NeuNAc represents *N*-acetylneuraminic acid.

Both diseases, with a recessive mode of inheritance, are caused by a disturbance in the turnover of the accumulated sphingolipids due to a genetically

induced deficiency in the catabolizing enzyme system, which resides in the lysosomes.

Cerebroside sulphates are catabolized by arylsulphatase A (i.e. cerebroside sulphatase) to give cerebroside and sulphate. The major monosialoganglioside is degraded by different glycosidases, which hydrolyse the different hexoses of the oligosaccharide chain one after the other.

Sulphatides are constituents of brain white matter including myelin. A deficient cerebroside sulphatase gives rise to the demyelinating disease metachromatic leukodystrophy. Gangliosides are constituents of the grey matter. The inability to catabolize the major monosialoganglioside or its lower homologues leads to swollen and disintegrated neurons, giving rise to infantile amaurotic idiocy. Three types of glycosidases (β -galactosidase, EC 3.2.1.23; *N*-acetyl- β -D-hexosaminidase, EC 3.2.1.30; β -glucosidase, EC 3.2.1.21) are involved in the catabolism of the major monosialoganglioside. Since each of these glycosidases can be deficient, different variants of the disease have been observed. They are characterized by more or less visceral involvement. Even the classical type of the infantile amaurotic idiocy (Tay-Sachs disease), with the accumulation of the trihexosyl ganglioside (Tay-Sachs ganglioside), caused by a deficient *N*-acetyl- β -D-hexosaminidase, shows three variations. They depend on the different patterns of two multiple forms of this enzyme that occur in humans.

Diagnostic and therapeutic possibilities of these inborn errors of metabolism will be discussed.

The Metabolism of Phosphatidylcholine in Brain Tissue

By G. B. ANSELL [*Department of Pharmacology (Preclinical), University of Birmingham Medical School, Birmingham B15 2TJ, U.K.*]

Phosphatidylcholine is found in all the subcellular components of the nervous system; small amounts of choline plasmalogen are also present. Variations in its fatty acid composition in different subcellular components, deriving from a mixture of neuronal and glial cells, are known in some detail (Kishimoto *et al.*, 1969; Sun & Horrocks, 1970). Differences in the fatty acid composition of neuronal and glial cells have also been studied (Fewster & Mead, 1968; Hamberger & Svennerholm, 1971).

Both the concentration of phosphatidylcholine and its fatty acid composition change during the development of the brain. Thus in the rat a maximum rate of deposition occurs during a short period in early life, such that the concentration in the whole brain of a 6-day-old rat is only 60% of that of a

20-day-old animal (Wells & Dittmer, 1967). However, as a percentage of the total phospholipid it actually decreases during development, a consequence of the more massive deposition of the so-called myelin phospholipids. During development the proportion of C_{18:0} and C_{18:1} acids increases at the expense of C_{16:0} and C_{16:1} acids (Kishimoto *et al.*, 1965).

Phosphatidylcholine is formed in animal tissues by the *N*-methylation of phosphatidylethanolamine, by the cytidine pathway and probably by a base-exchange reaction. Although the stepwise methylation of phosphatidylethanolamine does not occur in mammalian brain tissue, the enzymic components of the cytidine pathway have all been demonstrated. Choline kinase (EC 2.7.1.32) is present in the cell sap (McCaman & Cook, 1966; S. G. Spanner, unpublished work) and so is the choline phosphate cytidyltransferase (EC 2.7.7.15) (Chojnacki *et al.*, 1967), but the final stage, catalysed by choline phosphotransferase (EC 2.7.8.2), is probably largely microsomal (McCaman & Cook, 1966). The factors controlling the availability of the diglyceride acceptor remain unknown. In addition to this synthetic capacity of brain tissue, it is also likely that phosphatidylcholine is transferred intact from the blood to the brain (Hoelzl & Franck, 1969; Ansell & Spanner, 1971), though whether this is a means of transferring the phospholipid or simply choline is enigmatic.

Support for the significance of the cytidine pathway has come from investigations of the fate of intracerebrally injected [*Me*-¹⁴C]choline by Ansell & Spanner (1968), who demonstrated a rapid phosphorylation of the base and transfer to choline lipids, particularly phosphatidylcholine, though some incorporation by base exchange could not be eliminated. Labelled phosphatidylcholine was found in all subcellular fractions (Ansell & Spanner, 1970), and in 3 h there was a significant uptake into myelin of 18% of the total incorporation into brain choline lipids. From these results and those obtained with [1,2-¹⁴C]ethanolamine (Ansell & Spanner, 1967, 1968) it seems highly likely that there is a greater turnover of phospholipids in adult myelin than has hitherto been supposed. Confirmation has come from recent findings with intracerebrally injected [U-¹⁴C]-palmitate by Sun & Horrocks (1971) and labelled choline and other precursors by Jungalwala & Dawson (1971). It may be that intracerebral injection demonstrates a turnover of brain components that, for some reason, intraperitoneal injection of precursors does not (cf. Ramsey *et al.*, 1971).

A turnover of phosphatidylcholine in brain requires a catabolic process or an active removal of the lipid from the organ. Evidence for enzymes capable of hydrolysing phosphatidylcholine in brain at significant rates has been obtained by a number of

workers. Webster & Cooper (1968) and Cooper & Webster (1970) have clearly shown the presence of phospholipase A₁ (EC 3.1.1.4) and phospholipase A₂ in preparations of brain tissue active towards phosphatidylcholine; phospholipase A₁ is probably a microsomal enzyme and phospholipase A₂ is probably mitochondrial (Bazan, 1969). A lysophospholipase (EC 3.1.1.5) is also present in the particulate fraction (Leibovitz & Gatt, 1968), and the presence of a phospholipase C (EC 3.1.4.3) is indicated by the findings of Illingworth & Glover (1969). Ansell *et al.* (1971) have shown that a phospholipase C is present in the crude mitochondrial fraction of brain, does not require bivalent cations for activity and can release phosphorylcholine from phosphatidylcholine at a rate of about 100 nmol/h per g fresh wt. of tissue.

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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.

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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