The *N*-terminal sequence of the γ -chains has been studied in some greater detail, particularly for the allotypes A1 and A3 (Wilkinson, 1969a). The sequence of the first 34 residues has shown that there are 13 differences between the two sequences, and there are also a considerable number of positions that vary within each sequence; it is thus difficult to pinpoint those residues that may be responsible for the allotypic specificity. The sequence of the *C*-terminal half of the Fd region is known (Fruchter, Jackson, Mole & Porter, 1970), and as the serum pool used in this work was mainly of A1 specificity it may be taken as the A1 sequence; the corresponding sequence from A3 Fd fragment has been investigated in part and these data, taken with the peptide analyses of Koshland (1967), show that there is no allotype-related variation in this part of the sequence. A small amount of the remainder of the variable sequence has been investigated similarly and has so far failed to reveal further differences, but in view of the amino acid analysis data it is probable that more will be found. It would therefore seem that the amino acid sequence differences responsible for the *a* group specificities are present in the variable region of the γ -chain.

Preliminary results on the analysis and *N*-terminal sequence of α -chains of A1 and A3 specificity (Wilkinson, 1969b) suggest that similar allotype-related variations may be found in the α -chains. If this is true also of the μ -chains it would seem that the heavy chains of immunoglobulins share a similar set of variable sequences and that the constant parts of the sequence are class-specific.

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LIPID AND NEUROCHEMICAL GROUPS JOINT COLLOQUIUM ON 'CATABOLISM OF COMPLEX LIPIDS OF THE BRAIN'

Enzymic Hydrolysis of Glycolipids and Phospholipids

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The research effort in this laboratory in the recent few years has been directed mainly to the isolation and characterization of enzymes that catalyse the degradation of glycolipids and phospholipids. This resulted in partial purification of the enzymes that catalyse the complete degradation of sphingolipids of animal origin and of a plant β -galactosidase that hydrolyses monogalactosyl diglyceride. The pathways of total degradation of the sphingosine bases were elucidated and several enzymes that hydrolyse phospholipids were purified.

Degradation of glycosphingolipids. Five sphingolipid-hydrolysing enzymes were extracted from brain tissue, partially purified and characterized. These are: (1) neuraminidase; (2) β -*N*-acetylhexosaminidase; (3) β -galactosidase; (4) β -glucosidase; (5) ceramidase. The neuraminidase was tightly bound to particulate fractions and could not be solubilized; it was therefore employed as a protein dispersion in Triton X-100. It did not act on sialyl-

lactose or a sialic acid-rich glycoprotein, but utilized as substrates several gangliosides in which *N*-acetylneuraminic acid was linked to a terminal galactose or to a second sialic acid residue. *N*-Acetylneuraminic acid was not removed from gangliosides when located at a branch point (i.e. where both the sialic acid and *N*-acetylglucosamine were attached to galactose, as in G_{M1} and G_{M2} gangliosides). Three β -*N*-acetylhexosaminidases were isolated from brain. One, solubilized from particles, hydrolysed both *N*-acetylglucosaminides and *N*-acetylglactosaminides. The other two were purified from the 100000g supernatant; one hydrolysed *N*-acetylglucosaminides and the other hydrolysed *N*-acetylglactosaminides. Only the enzyme that was solubilized from the particles hydrolysed the *N*-acetylglactosaminidic linkages of the two glycolipids globoside and 'asialo Tay-Sachs ganglioside' (*N*-acetylglactosaminidogalactosidoglucosidoceramide). Tay-Sachs (or G_{M2}) ganglioside was hydrolysed at only about 2% of the rate of hydrolysis of the above two glycolipids. The brain β -galactosidase hydrolysed most glycosphingolipids in which the galactose residue was terminal. However, galactosylceramide and galactosylsphingosine, in which galactose is attached directly to the sphingosine base, were not attacked by this enzyme.

The β -glucosidase hydrolysed the glucosidic linkage of glucosylceramide, yielding ceramide (*N*-acylsphingosine). The amide linkage of this latter compound was hydrolysed to sphingosine and fatty acid by the enzyme ceramidase.

These five enzymes may account for the total degradation of the glycosphingolipids to their individual components. In the laboratory the individual enzymes were utilized for single-step hydrolyses of these glycolipids. It is, however, assumed that in the intact cell they act in unison and catalyse a multi-step successive degradation of the gangliosides and other complex glycosphingolipids to their individual components. These enzymes were isolated from a similar subcellular fraction of brain and have several similar properties (i.e. optimum pH values at about pH 5). Experiments are in progress to characterize the exact subcellular localization of these enzymes and test the hypothesis that they might reside on one membrane as part of a multi-enzyme complex that is responsible for degradation of compound lipids.

Metabolism of the sphingosine bases. Two enzymes that acylate the sphingosine bases were isolated. Ceramidase of rat brain, which hydrolysed the amide linkage of ceramide, also catalysed the reverse reaction, i.e. condensed sphingosine and a free fatty acid to form *N*-acylsphingosine. In the yeast *Hansenula ciferri*, an alternative acylation system was isolated. It transferred the acetyl moiety of acetyl-CoA to sphingosine bases or to their *N*-acetylated derivatives, yielding mono- or oligo-acetylated long-chain bases.

The pathways of total biodegradation of the sphingosine bases were elucidated. The bases were split between C-2 and C-3 (these carry the amino and secondary hydroxyl groups respectively) and the residue was oxidized. The products of these reactions were palmitic acid (with C₁₈ sphingosine or dihydro-sphingosine) or α -hydroxypalmitic acid (with phytosphingosine). The latter hydroxy acid was further decarboxylated by an α -hydroxy fatty acid decarboxylase, yielding pentadecanoic acid. Preliminary investigations on the latter enzyme showed that it could be partially purified by extraction from rat liver mitochondria and precipitation with ammonium sulphate. At this purification stage α -hydroxypalmitate was not decarboxylated unless the 100000g supernatant was added. The nature of these components is currently being investigated. Since either palmitic acid or pentadecanoic acid can be degraded and oxidized by the β -oxidation and tricarboxylic acid systems, these pathways, when coupled to those catalysed by the five hydrolases described above, account for the total degradation and oxidation of most animal glycosphingolipids.

Hydrolysis of plant glycolipids. α -Galactosidase and β -galactosidase were isolated from spinach

leaves and separated from each other. The β -galactosidase was purified about 1000-fold; it hydrolysed *p*-nitrophenyl β -galactoside at a rate of about 1000 μ mol/h per mg. It also hydrolysed the β -galactosidic linkage of monogalactosyl diglyceride at a rate of 2–4 μ mol/h per mg of protein. The α -galactosidase, which was purified only 15-fold, hydrolysed *p*-nitrophenyl α -galactoside at a rate of 6 μ mol/h per mg. At this purification stage the enzyme did not split the terminal α -galactosidic linkage of digalactosyl diglyceride.

Hydrolysis of phospholipids. The following phospholipases were extracted from brain tissue, solubilized and partially purified. Sphingomyelinase, an enzyme of the 'phospholipase C' type, hydrolysed sphingomyelin to ceramide (*N*-acylsphingosine) and phosphorylcholine, and ceramide phosphorylethanolamine to ceramide and phosphorylethanolamine. Sphingomyelin having sphingosine with the *erythro* configuration was hydrolysed five times as fast as that having sphingosine with the *threo* configuration. The enzyme had no lecithinase C activity, i.e. no phosphorylcholine and diglyceride were formed by action of this enzyme on lecithin (phosphatidylcholine). Partially purified preparations of sphingomyelinase also exhibited lecithinase A activity, splitting lecithin to a fatty acid and lysolecithin (1- or 2-acylglycerophosphorylcholine). By precipitating the sphingomyelinase at pH 5, the two activities could be separated from each other. The phospholipase A was then further purified and characterized as phospholipase A₁, i.e. an enzyme that hydrolyses the ester linkage in the α' (or 1)-position of phosphatidylcholine or phosphatidylethanolamine. The products of the action of this enzyme on glycerophosphatides were a predominantly-saturated fatty acid and 2-acylglycerophosphorylcholine or 2-acylglycerophosphorylethanolamine; these contained a predominantly-unsaturated fatty acid. The enzyme did not hydrolyse the ester bond in the β (or 2)-position of the phosphatides and was practically free of lysolecithinase activity. A lysolecithinase was subsequently extracted from rat brain; it had a relatively low molecular weight and could be freed of lecithinase activity. It hydrolysed the ester linkages of either 1- or 2-acylglycerophosphorylcholine, but not of the diacyl phosphatide. Experiments were recently conducted on brain 'plasmalogenase', i.e. an enzyme that split the vinyl ether linkage of plasmalogen (2-acyl-1-alkenylglycerophosphorylcholine). Purification of this enzyme and a study of its properties are currently in progress.

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