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BIOCHEMICAL STUDIES ON OVINE
CEROID-LIPOFUSCINOSIS

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ABSTRACT

The ceroid-lipofuscinoses are a group of inherited diseases of humans and animals characterised by brain atrophy and the storage of a fluorescent lipopigment in a variety of tissues. Clinical signs include loss of vision, seizures, mental retardation and dementia, with the disease culminating in premature death. Defects in lipid metabolism or the control of lipid peroxidation have been postulated to explain their pathogenesis. Specific defects in peroxidases, fatty acid metabolism, dolichol metabolism, retinol metabolism and iron metabolism have been proposed. Evidence for these mechanisms arises from the apparent lipid fluorescent Schiff base nature of the lipopigment.

This study reports the analysis of total tissue lipids and lipopigment isolated from tissues of sheep affected with ceroid-lipofuscinosis. Brain grey matter phospholipid fatty acids of diseased sheep were compared with those of normal sheep. Phosphatidylethanolamine of diseased sheep contained more 18:1(n-9) and less 22:6(n-3) than normal and their phosphatidylcholine less 16:0. Other differences were minor. No differences were found between the liver lipids nor the fatty acid profiles of their phosphatidylcholine, phosphatidylethanolamine or triglycerides.

Lipopigment from the liver of affected sheep was 70% proteinaceous, the rest being mainly lipids. These were only one sixth as fluorescent as total liver lipids, but contained a number of fluorophors. None were major components of the lipopigment or the postulated fluorescent product of lipid peroxidation. Lipopigment lipids included the lysosomal marker bis(monoacylglyceryl)phosphate that contained 42.9% linoleate and 16.5% linolenate. Lipopigment neutral lipids were dolichol, dolichyl esters, ubiquinone, free fatty acids and cholesterol, indicative of a lysosomal origin of the lipopigment. Phosphatidylcholine, phosphatidylinositol, phosphatidylserine and phosphatidylethanolamine were present in proportions and with fatty acid profiles typical of lysosomes.

Lipopigment isolated from liver, kidney, pancreas and brain of affected sheep without the use of proteolytic enzymes was two-thirds protein. Silver staining after sodium dodecyl sulphate polyacrylamide gel electrophoresis showed a major band of M_r 14,800, heterogeneous material between 5,000 - 9,000 M_r and a major band of $M_r < 3,500$. These components did not stain for RNA or carbohydrate, and were digested by a nuclease free protease. They are not normal lysosomal proteins. The presence of the 3,500 M_r proteins in whole affected tissue homogenates distinguished them from homogenates of normal tissue. Lipopigment levels of dolichol, ubiquinone and cholesterol were consistent with the lipopigment being protein enriched lysosome derived cytosomes.

The concentration of metals in lipopigment from the four tissues was also analysed. Liver lipopigment had a high copper content, 1.3%, kidney lipopigment a high iron content and brain lipopigment showed an accumulation of some trace elements. These data indicate that the lipopigment cytosomes have a history of being functional lysosomes as far as metal metabolism is concerned. Metal analyses of cerebrospinal fluid revealed no indication of a defect in metal metabolism.

It is concluded that ovine ceroid-lipofuscinosis is not a lipidosis, nor does the lipopigment arise from the abnormal peroxidation of lipids. There is no evidence of any disturbance in metal metabolism. Low molecular weight proteins are stored in lysosome derived organelles and on these grounds the ceroid-lipofuscinoses should be regarded as lysosomal proteinoses. These may result from defects in lysosomal proteolysis or its control.

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