indicates, however, that there is similar inhibition of isovaleryl-CoA dehydrogenase, for which a separate and specific existence has been proposed (Tanaka et al., 1972; Osmundsen et al., 1974). Similarly, the occurrence of octanoic acid in the urine in both syndromes indicates the inhibition of \( \beta \)-oxidation of other \( C_6-C_9 \) short-chain fatty acids, such acids not requiring acyltransferases to leave the mitochondria.

It is apparent from the present studies that the nature of the dicarboxylic aciduria may indicate which acyl dehydrogenases are being inhibited and may help in establishing the nature of the inhibitor. It is further apparent that some cases at least of Reye's-like syndrome in infants may be due to the toxic effects of analogues of hypoglycin and their organic acid metabolites and that similar causes may underlie other cases of dicarboxylic aciduria in infants. Elevated or abnormal dicarboxylic aciduria is therefore an important indicator of both defective \( \beta \)-oxidation and, in the presence of hypoglycaemia, of the possible ingestion of such toxic organic acids.

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The Location of Proteoglycans in Sheep Brain

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Studies from a number of laboratories have established the presence of glycosaminoglycans in tissues of the central nervous system. Comparatively little is known of the physiological role of these compounds in brain. However, some studies have indicated a possible involvement of these molecules in the storage of catecholamines in synaptosomes (Pycock et al., 1975). Until recently glycosaminoglycans have been chemically characterized as discrete chains, although the presence of xylosyl residues at the potentially reducing ends has been suggested (Margolis et al., 1972). We have reported the occurrence of both chondroitin sulphate and heparan sulphate proteoglycans in whole sheep brain tissue by using a dissociative method of extraction (Branford White & Hudson, 1977; Branford White & Hardy, 1977). Both these proteoglycans were found to exist as multi-chained complexes in which the glycosaminoglycan chains are covalently linked to a protein core. The protein moieties differed in amino acid composition and immunological activity. To obtain a greater understanding of the possible role of these macromolecules in brain tissue, we have extended this study by characterizing their distribution in specific areas within the brain.
Sheep brains obtained 1 h after death were freed from adhering tissue and the brains were carefully dissected into cerebrum, cerebellum and brain stem. After extraction with chloroform/methanol (Branford White & Hudson, 1977), lipid-free fractions accounted for 10% of the original wet weight of each brain fraction. However, the hexuronic acid content of the lipid-free fractions did vary; cerebrum contained 0.56% and cerebellum 0.18% and brain stem 2.0% of dry weight. Batches were extracted with 4M-guanidinium chloride. On removal of guanidinium ions by dialysis the residue was extracted with 0.4M-citrate buffer, pH 4.5. This extract was chromatographed on DEAE-cellulose (Whatman) and the column eluted with a linear gradient (0--2M-NaCl) in 0.1M-citrate buffer, pH 4.5. Two major hexuronic acid-containing fractions were eluted, at 0.5M-NaCl (fraction B1) and 0.72M-NaCl (fraction B2) respectively. Furthermore, when chromatographed on 4% agarose both fractions B1 and B2 appeared to behave as monodispersed species in which hexuronic acid and protein-containing fractions migrated as single bands. Amino sugar analysis revealed that fraction B1 contained only galactosamine. Moreover, treatment with chondroitinase ABC (EC 4.2.2.4) resulted in degradation of glycosaminoglycan chains, which were detected after gel filtration on Sephadex G-25 (Pharmacia). Protein-containing fractions were excluded from the column, whereas hexuronic acid-containing fractions were retarded. All B2 fractions were found to be undegraded by the enzyme and only glucosamine was observed on analysis.

The distribution and chemical composition of fractions B1 and B2 in the various brain fractions are summarized in Table 1. All fractions were found to be highly sulphated. However, on treatment with nitrous acid, formation of the anhydromannose derivative was only shown in the B2 fractions. Moreover, fraction B2 from cerebellum contained about 7% more N-sulphated residues than cerebrum and brain stem. All B2 fractions contained the same degree of sulphate substitution, which suggests that the mechanism attributed to O- and N-sulphation may vary depending on the site of synthesis in the brain.

Fractions were further characterized by the formation of a Toluidine Blue–glycosaminoglycan complex (Jacques & Wollin, 1967). Absorption maxima of 540 and 521 nm were obtained for B1 and B2 fractions respectively (Fig. 1). It would appear that the presence of the protein moiety interferes with the formation of the complex when compared with standard glycosaminoglycan chains.

The results presented here show that chondroitin sulphate and heparan sulphate proteoglycans are not evenly distributed in brain tissue. Cerebrum contains 88% of the total amount of proteochondroitin sulphate present in whole brain, whereas 60% of the proteoglycan in brain stem is proteoheparan sulphate. Similar findings have been reported in which chondroitin sulphate was found to be the most dominant glycosaminoglycan isolated from neural perikarya and astrocytes from cerebrum of rat brain (Margolis & Margolis, 1974).

Although the function of proteoglycans in neural tissue is still not clear, molecular-weight studies have implied that proteochondroitin sulphate may be located on the cell

| Table 1. Chemical composition and distribution of proteoglycans in brain |
|----------------|----------------|----------------|----------------|----------------|
| Fraction      | Protein (% of dry wt. of sample) | Hexosamine: hexuronic acid (molar ratio) | Sulphate: hexosamine (molar ratio) | Yield (% dry lipid-free tissue) |
| Cerebrum B1   | 20.8                              | 1.1                                   | 1.1                        | 0.38                        |
| Cerebrum B2   | 12.1                              | 1.2                                   | 1.7                        | 0.20                        |
| Cerebellum B1 | 21.6                              | 1.0                                   | 0.9                        | 0.21                        |
| Cerebellum B2 | 11.8                              | 0.9                                   | 1.8                        | 0.17                        |
| Brain stem B1 | 20.5                              | 1.0                                   | 1.0                        | 0.15                        |
| Brain stem B2 | 12.0                              | 1.1                                   | 1.8                        | 1.7                         |
Fig. 1. Absorption spectra of Toluidine Blue–glycosaminoglycan complexes

Glycosaminoglycan samples (1 mg/ml) in 0.05 M-barbital buffer, pH 8.6, were mixed with an equal volume of Toluidine Blue (100 μg/ml) and the spectrum was scanned in a Cecil CE 505 double-beam spectrophotometer. Traces of fractions B1 (a) and B2 (b) were compared against chondroitin sulphate (c) and heparan sulphate (d) standards.

surface or in an intercellular environment (Branford White & Hudson, 1977). Furthermore, Ca2+ interactions have been implicated with these macromolecules (Wang & Adey, 1969). Therefore distribution of proteoglycans may influence the movement and binding of this cation within specific parts of the brain.


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Differential Effects of D- and L- Allylglycine (2-Aminopent-4-enoic Acid) on Regional Cerebral γ-Aminobutyrate Concentrations

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Experimental evidence indicates a decrease in cerebral γ-aminobutyrate concentration, after inhibition of glutamate decarboxylase (EC 4.1.1.15) activity, as a critical factor in the mechanisms of the convulsant action of allylglycine (Alberici de Canal et al., 1969; Horton & Meldrum, 1973; Fisher & Davies, 1974). However, anomalies between the inhibition of glutamate decarboxylase activity by allylglycine in vivo and in vitro led us to suggest that the formation of a metabolite of allylglycine in vivo was responsible for the inhibition of glutamate decarboxylase. Orlowski et al. (1977) demonstrated that both D- and L-isomers of allylglycine were convulsant, L-allylglycine being 3–4 times as