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Immunological Identity of Human Liver Hexosaminidases A and B

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The relationships of the two forms of *N*-acetyl- β -hexosaminidase commonly found in human tissues are uncertain. Hexosaminidase A appears to be required for breakdown of ganglioside GM₂, as evidenced by the effects of its absence in Tay-Sachs disease (O'Brien *et al.*, 1971).

In these cases hexosaminidase B is present in normal or elevated amounts. A few exceptional cases have been reported of the complete deletion of both forms (Sandhoff *et al.*, 1968), but to date no specific disorder associated with lack of hexosaminidase B alone has been described.

In order to examine more closely the biochemical relationships of these two enzyme we have raised antisera to each isoenzyme and studied their cross-specificity.

Enzymes were prepared from human liver by ammonium sulphate fractionation, ion-exchange cellulose and Sephadex G-200 chromatography and isoelectric focusing. The products showed about 50-fold increase in specific activity over that of the starting material, and gel electrophoresis indicated that neither antigenic fraction was homogeneous.

Both preparations evoked a good immune response when injected into rabbits, and the antisera so prepared precipitated the enzyme in double-diffusion and immunoelectrophoresis experiments as an enzymically active arc that could be stained with histochemical substrates (Pugh & Walker, 1961).

Complete cross-reaction was observed. Both sera gave an enzymically active arc with purified hexosaminidase A, but the amount of precipitin in this case was too small to give a visible arc when protein stains were used. Both isoenzymes were completely removed from solution by either antisera. The antiserum raised to liver enzyme cross-reacts with the enzyme from kidney, spleen and brain, the precipitated enzyme remaining fully active in each case.

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Preparation of Monomeric Horse Spleen Ferritin by Gel Filtration

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It has been previously shown (Williams *et al.*, 1966; Zamiri & Mason, 1968) that ferritin contains oligomers, usually, in horse spleen ferritin, making up 16–18% of the total molecules. Although the stability of ferritin oligomers has been disputed (Richter & Walker, 1967), they have proved resistant to a variety of reagents and conditions (Williams & Harrison, 1968). Since ferritin is such an important protein marker in cell biology, it was thought useful to investigate again the stability of ferritin oligomers in order to see whether a purified monomeric preparation could be made. Such a purified preparation would greatly enhance quantitative studies with free ferritin and with ferritin-labelled antibody (Voak & Williams, 1971). Various commercial horse spleen ferritin preparations were run on Sepharose 4B columns (76cm $\times$ 3cm). The $E_{280}^{1\text{cm}}$ of the eluent fractions was measured and the front and rear thirds of the peak were reconcentrated by ultracentrifugation and recycled. Counts of at least 500 iron cores/fraction were made from electron micrographs (Williams & Harrison, 1968) and the percentage composition of monomers and oligomers of each fraction was calculated. From these results it was found that the overall proportions of monomer and oligomer before and after gel filtration were closely similar, i.e. despite the dilution involved in gel filtration the oligomers had remained stable. Purified monomer fractions showed no aggregation of molecules during storage over several weeks at 4°C. On first passage through Sepharose 4B, all oligomers larger than dimers were eliminated. In some preparations large aggregates were separated and eliminated. More than 40% of the ferritin occurred in fractions made up of at least 95% monomer. The tail fractions contained more than 99% monomer. When the rear one-third of the peak was recycled, 50–60% of ferritin was contained in fractions with at least 95% monomer.

These monomer-enriched ferritin preparations enhance the use of ferritin in studies on protein penetration and pinocytosis. In studies with ferritin-labelled antibody the use of monomer-enriched ferritin greatly facilitates the quantitative determination