activity (μmol of maltotriose liberated from pullulan/min per mg dry wt.) of the cells reached maximum values of 0.25–0.30 unit at a dilution (growth) rate (D) of 0.2h^{-1} at 30°C at pH 7.1. In other nutrient-limited conditions the activity was negligible (cf. Bender & Wallenfels, 1961). Intact and disrupted cells had similar activities, in general, and in pullulan-limited conditions at $D = 0.2\text{h}^{-1}$ the specific activity observed is in considerable excess of that required to hydrolyse the carbon substrate at the rate necessary to maintain growth. Culturing of maltose-adapted cells in amylopectin-limited medium gave higher activities, which reached a maximum value of 0.45 unit at $D = 0.35\text{h}^{-1}$, but growth could not be maintained in glycogen-limited conditions even at $D = 0.1\text{h}^{-1}$.

Fifteen other N.C.I.B. strains of *K. aerogenes* have also been examined in maltose-limited medium at $D = 0.2\text{h}^{-1}$. Strains N.C.I.B. 5938 and N.C.I.B. 8017 had pullulanase activities of 0.86 and 0.83 unit respectively, strains N.C.I.B. 8153 and N.C.I.B. 9146 showed negligible activity even after disruption (but were inducible with pullulan), and the others were similar to strain N.C.I.B. 418.

Although understandable in cells utilizing pullulan and amylopectin, the function of pullulanase in maltose-utilizing cells is not clear. Abdullah & French (1966) report that, besides hydrolysing oligosaccharides, pullulanase can synthesize an α -1,6-dimer and even a trimer from maltose. The formation of polysaccharides in this way provides a possible function. However, no clear correlation is apparent between the total polysaccharide content of cells and their pullulanase activity.

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Separation of α -L-Fucosidases in Extracts of *Trichomonas foetus*

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The destruction of human blood-group H and Le^a serological activity by extracts of the protozoan *Trichomonas foetus* has been attributed to the action of two different α -L-fucosidases (Tyler & Watkins, 1960; Watkins, 1962). Electrophoresis of the crude *T. foetus* extracts on starch columns gave partial separation of the enzymes destroying blood-group

H and Le^a activities; from their action on low-molecular-weight oligosaccharides, together with the known structures of the blood-group H and Le^a determinants (cf. Watkins, 1966), the enzymes were tentatively characterized as α -1,2- and α -1,4-L-fucosidase respectively. This communication reports attempts to further purify and characterize the enzyme that destroys blood-group Le^a activity.

Extracts of the organisms, prepared as described previously (Watkins, 1966), were fractionated with ammonium sulphate and the material that was precipitated at 55–80% saturation was, after dialysis, subjected to starch column electrophoresis on an LKB Uniphor apparatus at 8V/cm at pH 6.2 for 24h. A single peak with H-destroying activity was eluted from the column, followed by a peak with Le^a -destroying activity. The fractions with Le^a -destroying activity were pooled, dialysed, concentrated and applied to a DEAE-cellulose (Whatman DE52) column in 0.04M-phosphate buffer, pH 6.0. Elution with a linear salt gradient (0–1.0M-NaCl in 0.04M-phosphate buffer, pH 6.0) separated the Le^a -destroying enzyme from a small amount of residual H-destroying enzyme.

Tested on low-molecular-weight substrates, the Le^a -destroying enzyme preparation released fucose from lacto-*N*-fucopentaose II, in which the fucose is attached by an α -(1 $\rightarrow$ 4)-linkage to *N*-acetylglucosamine, but did not hydrolyse lacto-*N*-fucopentaose I or 2'-fucosyl-lactose, in which the fucose is linked α -(1 $\rightarrow$ 2) to galactose (cf. Kuhn, 1957). Although free from α -1,2-L-fucosidase activity, the Le^a -destroying enzyme preparation released fucose from 3-fucosyl-lactose (cf. Montreuil, 1960), lactodifucotetraose and lacto-*N*-difucohexaose I (cf. Kuhn 1957), results that indicate the presence of an α -1,3-L-fucosidase in the preparation in addition to the α -1,4-L-fucosidase. Whether these two activities are both associated with the same enzyme, or result from two different enzymes in the preparation, remains to be established. The H-destroying enzyme, separated from the Le^a -destroying enzyme by DEAE-cellulose chromatography, hydrolysed α -(1 $\rightarrow$ 2)-fucosyl linkages in low-molecular-weight substrates, but had no action on α -(1 $\rightarrow$ 3)- or α -(1 $\rightarrow$ 4)-linkages.

The Le^a -destroying enzyme destroys both blood-group Le^a and blood-group Le^b activities in glycoproteins. When tested on blood-group-H/ Le^b -active glycoproteins the decrease in blood-group Le^b activity is accompanied by an enhancement of blood-group H activity. The blood-group Le^b determinant is a difucosyl structure formed as an interaction product of the H and *Le* blood-group genes (Marr *et al.* 1967); removal of the α -(1 $\rightarrow$ 4)-linked fucose by the Le^a -destroying enzyme would therefore be expected to result in a loss of blood-group Le^b activity and the exposure of blood-group-H-active groupings.

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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