We thank Dr. A. Gauhe and Dr. J. Montreuil for gifts of oligosaccharides and the Microbiological Research Establishment, Porton Down, Wilts, U.K., for the growth of the *T. foetus* organisms.

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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

of antigenic sites and the recognition of antigen patterns on cell surfaces.

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Hydroxylation of Fatty Acids by Avocado Supernatant Fraction

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Preparations from the avocado (*Persea americana*) mesocarp rapidly synthesize fatty acids from C₂ precursors. Yang & Stumpf (1965) first showed the incorporation of radioactivity from [¹⁴C]malonate and [¹⁴C]malonyl-CoA into long-chain fatty acids by the 15000g supernatant. This latter activity is due to soluble proteins rather than microsomal particles. However, the 105000g supernatant is unable to elongate medium-chain fatty acids (C₈-C₁₂) in the presence of added malonyl-CoA and appropriate cofactors (NADPH, acyl-carrier protein, GSH).

When fatty acids (C₈-C₁₂) were incubated with the 105000g supernatant under conditions used for fatty acid synthesis (Harwood & Stumpf, 1971) a hydroxy acid was produced. The possibility of the hydroxy fatty acid being α -hydroxy or ω -hydroxy was eliminated by chemical degradation and chromatographic analysis. The product co-chromatographed as the methyl ester or trimethylsilyl ether on g.l.c. and t.l.c. in two separate systems with the β -hydroxy derivative of the substrate. Butyrate, heptanoate, myristate, palmitate and stearate are not hydroxylated.

The reaction requires CoA, ATP, acyl-carrier protein and molecular O₂. The chain-length specificity is not due to the acid-CoA ligase, since myristyl-CoA is not hydroxylated. It does not take place via β -oxidation because (a) no radioactivity was incorporated from ³H₂O under the reaction conditions, (b) no loss of radioactivity from 1-¹⁴C-labelled medium-chain fatty acids took place during the incubation and (c) the observed specificity would not have occurred since β -oxidation utilizes long-chain acids as well.

The hydroxylating enzyme is sensitive to iodoacetamide and, consequently, is protected by GSH. Production of the β -hydroxy acid is diminished in the presence of NADH and/or malonyl-CoA. This is probably due to the production of NAD⁺, since

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addition of the latter (at 0.5mM) completely inhibits the reaction. Slight inhibition of the reaction was achieved with cyanide (0.1mM); 5,5'-bipyridyl (1 μ M) or CO (CO+O₂, 83:17) gave strong inhibition, thus suggesting that a heavy-metal ion (such as Fe²⁺) is involved in the hydroxylation. Because of the soluble nature of the avocado hydroxylating system it should be possible to purify the enzyme and study the exact mechanism of hydroxylation.

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Low-Fat Diets and the Survival of Newborn Rats

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Phospholipids and protein are the major components of cellular and subcellular membranes. In animal tissues the essential fatty acids and their long-chain metabolic derivatives are consistently found as the major fatty acids in phospholipids.

In the rat essential-fatty acid deficiency affects reproduction: deficient females frequently abort or the pups die soon after birth; the male rats are generally sterile and will not mate (Holman, 1968). Deficiency experiments are frequently carried out by using diets completely free from fat; however, this is a situation most unlikely to occur in Nature. We examined rats fed on a low-fat diet (0.6% fat; 10g of diet contained 11mg and 2.2mg of linoleate and linolenate respectively) from weaning to maturity; on this diet the rats did not develop markedly scaly tails and the eicosatrienoate/arachidonate ratio in their liver phospholipids was only 0.1. Female rats on this diet were mated and only about 12% of the pups (13 litters) survived longer than 2 weeks. The same diet with 6% fat added isocalorically (10g of diet contained 211mg and 43mg of linoleate and linolenate respectively; control diet) gave a survival rate greater than 50% (12 litters).

Tissues were analysed within 24h of birth from 11 randomly selected pups on the low-fat diet and nine on the control diet. On the low-fat diet the pups were 27% smaller and there was significantly less lipid in their livers (24.3 $\pm$ 2.3mg/g compared with 45.6 $\pm$ 4.7mg/g). The percentage of *n*-6 and *n*-3 unsaturated fatty acids in the liver lipids was decreased by 50% and the eicosatrienoate/arachidonate ratio was 0.47. The pups of the mothers given this diet had smaller brains (0.20 $\pm$ 0.01g compared with 0.29 $\pm$ 0.01g) and the polyenoic fatty acid content was decreased by 20%. The fatty acid composition had not altered as