Electrophoretic mobility was measured at 37°C as previously described (Delpech et al., 1980). Insulin (1 munit/ml) was added at zero time. Dashed lines indicate measurements in the presence of neuraminidase (0.2 pg/ml) added at zero time. Dashed lines indicate measurements in the presence of neuraminidase (0.2 pg/ml) added at (a) zero time and (b) 10 min after the addition of insulin.

insulin has been suggested by Cuatrecasas & Illiano (1971) on the basis of neuraminidase treatment of adipose-tissue cells. Enzymic digestion was reported to have no effect on the total quantity of insulin receptor in the membranes or on the affinity of the receptor for insulin, but abolished the effect of insulin on glucose transport and on lipolysis. As shown in Fig. 1, neuraminidase action inhibits the observed insulin effects on chick erythrocyte mobility, and this would be consistent with the suggestion of a dissociation of the electrophoretic effects and receptor-binding properties of the molecule.

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**Multiple forms of membrane-bound β-glucosidase in human leucocytes**

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Leucocytes are commonly used for the enzymatic diagnosis of Gaucher’s disease, a sphingolipidosis characterized by a deficiency of the lysosomal enzyme β-glucosidase (glucocerebrosidase) (Snyder & Brady, 1969). The enzyme appears to be present as two components, one with optimal activity at about pH 4.5, the other at pH 5.0–5.5 (Beutler & Kuhl, 1970; Turner et al., 1977; Butterworth & Broadhead, 1978). We have studied further the membrane-bound β-glucosidase in leucocytes from controls, heterozygotes for and patients with type-1 (adult-onset) Gaucher’s disease. The results provide evidence for biochemical and genetic heterogeneity in this disorder.

Leucocyte homogenates were prepared in distilled water and centrifuged (bench centrifuge 2500 rev./min, 10 min) to obtain a crude membrane fraction as the source of enzyme. Thermobility studies, and assays of β-glucosidase activity in phosphate/citric acid buffer, were as described previously (Yaqoob & Carroll, 1980).

The activity of membrane-bound β-glucosidase in control leucocytes was maximal at pH 5.5–6.0 ('pH 5.5-activity'), with a pronounced shoulder on the curve at pH 4.5 ('pH 4.5-activity'), as was noted previously for the total activity of leucocyte homogenates (Beutler & Kuhl, 1970). The peak activity at pH 5.5–6.0 does not represent the soluble β-glucosidase, which is a minor component in leucocytes (it comprised about 20% of the total activity of the homogenate, assayed at pH 5.5). The pH 4.5-activity and pH 5.5-activity had identical K_m values (2.0 mM with the 4-methylumbelliferyl β-D-glucopyranoside substrate), but the latter was the more active of the two: the V_max values were 5.0 and 10 nmol/l per mg of protein respectively. Both enzymes rapidly lost activity at 52.5°C, the pH 5.5-enzyme being slightly more stable (20–30% residual activity after 1 h) compared with the pH 4.5-enzyme (10–20% residual activity). Taurocholate (up to 3.0 mg/ml) inhibited both enzymes, although the pH 5.5-activity was somewhat less susceptible (43% inhibition at a detergent concentration of 2.0 mg/ml) than the pH 4.5-activity (60% inhibition).

The activity of membrane-bound β-glucosidase in leucocytes, assayed at pH 4.5 and pH 5.5, was compared in controls, heterozygotes for and patients with type-1 Gaucher’s disease. In one experiment, Gaucher’s-disease patient 1 was found to have no detectable activity at pH 4.5, but approximately normal activity at pH 5.5. His parents (obligate heterozygotes for and patients with type-1 Gaucher’s disease) had normal activity at both pH 4.5 and pH 5.5. Gaucher’s disease patients: 2, 3, 4, 5 (1.79–2.32) (4.10–5.09) (2.42–3.84) (5.47–7.46) (2.0 mg/ml) 32.1 (2.42–3.84) (5.47–7.46) 1.58 (1.07–2.32) (4.56–10.5) 0 0 2.11 (1.79–2.32) (4.10–5.09) 0.98 (0.96–1.00) (1.27–1.38) 0 0.31 0.30 0.35 0.95

Table 1. Activity of membrane-bound β-glucosidase in leucocytes from controls, heterozygotes for and patients with type-1 Gaucher’s disease

Specific activity is expressed as nmol/l per mg of protein. Leucocyte homogenates had been stored at −20°C for 1–3 months (upper set of values) or 6–15 months (lower set).
heterozygotes) and two siblings (both heterozygotes; E. Young, personal communication) had a mean pH 4.5-activity that was 49% of that of the controls; their pH 5.5-activity was normal, as was to be expected. These results indicate that the genetic lesion in this family affects only the membrane-bound β-glucosidase activity at pH 4.5: presumably this enzyme is identical with glucocerebrosidase. The disorder is referred to subsequently as variant A of type-1 Gaucher's disease.

In another experiment, the mean activity of membrane-bound β-glucosidase in control leucocytes was somewhat lower than that found previously (Table 1). This is probably a result of the prolonged storage of the homogenates. However, the ratio of activity at pH 5.5 to that at pH 4.5 remained constant at about 2:1. Of the four unrelated Gaucher's-disease patients studied, two had no detectable β-glucosidase activity at either pH, whereas two had residual activity (about 16% of that of controls) at each pH. These biochemically distinct disorders are referred to as variant B and C respectively. Two heterozygotes had intermediate activity at both pH 4.5 and pH 5.5. The pH 4.5-enzyme of the one patient studied was remarkably thermostable, as is the membrane-bound β-glucosidase from spleen of Gaucher's-disease patients (Yaqoob & Carroll, 1980).

It thus appears that there may be as many as three different genetic lesions (variants A, B and C) that are expressed phenotypically as Gaucher's disease. Furthermore, the β-glucosidase activities at pH 4.5 and pH 5.5 seem to represent separate enzymes that nevertheless have a structural and genetic interrelationship. This apparent biochemical and genetic heterogeneity in type-1 Gaucher's disease could be investigated further by analysis of possible intergenic complementation between the mutant cells.

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The distribution of four peptide hydrolases along the small intestine of the adult human

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In contrast with the extensive data on the distribution of di-saccharidase enzymes along the human gut, relatively little is known about the peptidases. This may be explained partly by the difficulty of obtaining fresh samples of human tissue, and by the confusion arising from the broad substrate specificity of many peptidases against the wide range of available synthetic peptides. Recently, six peptidases have been identified in human intestinal mucosa (Sterchi & Woody, 1980a) and of these the Enzyme Commission recognises four: aminopeptidase A (EC 3.4.11.7), aminopeptidase M (EC 3.4.11.2), dipeptidyl-peptidase IV (EC 3.4.14.2) and γ-glutamyltranspeptidase (EC 2.3.2.2).

We now report the distribution of these four peptidases, together with that of sucrase (a marker for brush-border membrane) along an entire small intestine that had been obtained from a 29-year-old male kidney donor. In addition, the distribution of enzymic activity between the high-speed supernatant and particulate fraction was investigated.

The small intestine was removed from the donor immediately after death and cut into 17 sections, 30 cm long, which were then rinsed through with 0.15 m-NaCl at 4°C. Each piece of gut was drained, wrapped in aluminium foil and frozen with solid CO2. After 8 weeks storage at −20°C, a 2 cm section was cut off the distal end of each piece. The mucosa was scraped away with a scalpel and homogenized at a final concentration of 2.5% (w/v) in 2 mM-Tris/50 mM-mannitol buffer, pH 7.4. A 5 ml portion of the homogenate was centrifuged at 100,000 g for 1 h. The supernatant was retained and the pellet was resuspended in 5 ml of Tris/mannitol buffer. Protein was measured in the original homogenate, supernatant and pellet by the method of Lowry et al. (1951). Sucrase activity was determined by the method of Dahlqvist (1968). Peptidases were assayed with the fluorimetric method described by Sterchi & Woody (1980b). Results are expressed as µmol of substrate hydrolysed/min per g of protein at 37°C.

As shown in Fig. 1, the specific activity of the particulate form of aminopeptidase A increases approx. 6-fold from 1.9 units/g of protein near the pylorus to 11.0 units/g of protein at the ileo-caecal junction. Similarly, dipeptidyl-peptidase IV activity in the particulate fraction increases approx. 3-fold along the length of the gut from 2.07 to 5.95 units/g of protein. In contrast with these findings, the specific activity of the particulate form of aminopeptidase M varies from

![Fig. 1. Distribution of (a) aminopeptidase A and (b) dipeptidyl-peptidase IV along the human small intestine](image)

Enzymic activity is expressed as µmol of substrate hydrolysed/min per g of protein. 

![Fig. 2. Distribution of (a) aminopeptidase M and (b) sucrase along the human small intestine](image)

Details as in Fig. 1.

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