The Separation and Characterization of the Methylumbelliferyl 
β-Galactosidases of Human Liver

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1. A previously uncharacterized form of human liver acid β-galactosidase (EC 3.2.1.23), 
possibly a dimer of molecular weight 160000, was resolved by gel filtration. It has 
the same ability to hydrolyse GM₁ ganglioside as the two other acid β-galactosidase forms. 
2. The low-molecular-weight forms of acid β-galactosidase undergo salt-dependent 
aggregation. 3. The high-molecular-weight component may consist of the low-molecular-
weight forms bound to membrane fragments. It can be converted completely into 
a mixture of these forms. 4. The neutral β-galactosidase activity can be resolved into two 
forms by DEAE-cellulose chromatography. They differ in their response to Cl⁻ ions. 5. 
A new nomenclature is suggested for the six β-galactosidases so far found in human liver. 
6. The enzymic constituents of the β-galactosidase bands resolved by electrophoresis were 
re-examined. The A band contains three components. A two-dimensional electrophoretic 
procedure for resolving the A band is described. 7. The effect of neuraminidase treatment on 
the behaviour of β-galactosidases in various separation systems is examined.

There are at least three types of enzymes in human liver able to hydrolyse methylumbelliferyl β-D-
galactoside (Ockerman & Hultberg, 1968; Van Hoof & Hers, 1968; Ho & O'Brien, 1969). The 'acid'
β-galactosidases, which are optimally active in the pH region 4.0–4.5, show the highest specific 
activities. These are stimulated and stabilized by Cl⁻ ions and are inactive towards methylumbelliferyl-
β-D-glucoside (Ho & O'Brien, 1971). The 'neutral' 
β-galactosidase activity, with pH optimum close to 
6.0, will also hydrolyse β-D-glucosides and, to a lesser 
extent, α-L-arabinosides and β-D-xylosides. It shows a 
net inhibition by Cl⁻ ions. Little is known of the 
'alkaline' β-galactosidase, which appears to have a 
relatively low activity and pH optimum in the region of 
PH8.5 (Van Hoof & Hers, 1968).

The most commonly used methods of separating human liver β-galactosidases have been Sephadex-
gel filtration (Hultberg & Ockerman, 1969; Butterworth et al., 1972; Li et al., 1974) and gel electrophoresis (Ho & O'Brien, 1971), although the use of 
isoelectric focusing (Hultberg & Ockerman, 1969; 
Suzuki & Suzuki, 1974) and ion-exchange chromatography (Norden et al., 1974) has also been reported. 
In previous work gel filtration has resolved two 'acid'
forms, having apparent molecular weights of 80000 
and higher than 200000 respectively, and a single 
'neutral' form of molecular weight about 40000. 
The lower-molecular-weight 'acid' β-galactosidase 

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named β-galactosidase A1, A2 and A3 in order of increasing molecular weight. Evidence presented in this paper shows that the 'neutral' activity can be resolved into two forms by DEAE-cellulose chromatography. These forms are genetically and structurally distinguished from the 'acid' forms and are therefore called β-galactosidase B1 and B2 in order of elution. If the 'alkaline' activity is shown to be distinct from the B forms it could be called β-galactosidase C.

An accurate knowledge of the relationship between human β-galactosidases is particularly important in the study and diagnosis of GM1 gangliosidosis and any other diseases that arise from β-galactosidase deficiencies. Assay of activity towards the substrate that accumulates in the diseased tissues is often relatively slow and difficult, so that it is preferable to substitute an artificial substrate, combined, if necessary, with a rapid separation technique, such as gel electrophoresis. The relationships between the three bands obtained on starch-gel electrophoresis (Ho & O'Brien, 1971) and the isoenzymic forms of β-galactosidase obtained by other separation techniques have therefore been investigated and clarified.

Methods

Glycosidase assays

The enzyme preparation (0.1 ml) was incubated for 30 min at 37°C with 0.5 ml of 1 mM-methylumbelliferyl β-D-glucoside or galactoside (Koch-Light Laboratories, Colnbrook, Bucks., U.K.) dissolved in McIlvaine 0.1 M-sodium phosphate/0.2 M-citric acid buffer at pH 4.35 or pH 6.0. More active partially purified preparations were assayed by incubating 10 or 20 μl with the same volume of buffered substrate for 15 min. In either case the reaction was terminated with 3.0 ml of 0.1 M-glycine/NaOH buffer, pH 10.4. Fluorescence was measured at 440 nm in a Locarte LF5 fluorimeter with LF2 filter on the activation side. In determinations of pH optima a 2 mM substrate solution in water was prepared and diluted 1:1 (v/v) with McIlvaine buffers of the appropriate pH range.

GM1 β-galactosidase was assayed as described by Ho et al. (1973).

Protein assay

Protein was assayed by the Folin method (Lowry et al., 1951), with bovine serum albumin as standard.

Gel filtration

Sephadex G-150 or G-200 [fine grade; Pharmacia (G.B.) Ltd., London W5, U.K.] was swollen in 10 mM-sodium phosphate buffer, pH 6.0, containing 5 mM-NaCl, by heating for 5 h at 90–100°C. Jacketed columns (40 cm × 2.5 cm diam. or 100 cm × 2.5 cm diam.; Pharmacia) were packed under gravity and eluted with the same buffer. The loading and elution of enzyme samples (2–3 ml) was carried out at 4°C with a flow rate of 21 ml/h. Fractions (2.4 or 3.6 ml according to the column size) were collected in a BTL Chromofrac system (Baird and Tatlock, Chadwell Heath, Essex, U.K.) maintained at 4°C. The columns were calibrated by the methods of Andrews (1970) by using Blue Dextran (mol.wt. 2 × 10^6), bovine serum albumin (6.7 × 10^5), horse-radish peroxidase (4.9 × 10^4) (Sigma, Kingston-upon-Thames, Surrey, U.K.) and cytochrome c (1.2 × 10^4) (BDH, Poole, Dorset, U.K.) as markers.

Ion-exchange chromatography

DEAE-cellulose DE 23 (Whatman Chemical Co., Maidstone, Kent, U.K.) was equilibrated in 10 mM-sodium phosphate buffer, pH 6.0. For analytical-scale separations (up to 2 ml of enzyme preparation) it was packed into the barrel of a 2 ml syringe at room temperature (17–20°C), and for preparative scale (up to 40 ml) into a jacketed column (40 cm × 1 cm diam.) at 4°C. Columns were eluted at 60 ml/h with several volumes of the same buffer and then with this buffer containing a linear concentration gradient (0–0.3 M) of NaCl. Fractions (1.5 or 3.6 ml) were collected. Columns and eluted fractions were maintained at 4°C.

Starch-gel electrophoresis

Starch gels were prepared from hydrolysed starch (Connaught Medical Research Laboratories, University of Toronto, Toronto, Ont., Canada), suspended in 2.3 mM-sodium phosphate buffer, pH 7.0, and allowed to set in Perspex trays (22 cm × 13 cm × 0.8 cm). Enzyme samples were inserted on Whatman 3 MM filter paper at an origin line 12 cm from one end of the tray. The gel was covered with a thin polyester sheet and connected via 3 MM filter paper to buffer vessels containing 50 mM-sodium phosphate buffer, pH 7.0. After electrophoresis at 4°C for 3.5 h at 200 V and 40 mA the gels were cut transversely and the freshly exposed surfaces covered with 3 MM filter paper soaked in the appropriate methylumbelliferyl substrate. Fluorescent bands were visible within 10 min and were intensified with 3 MM filter paper soaked in 0.1 M-glycine/NaOH buffer, pH 10.4. For greater resolution the substrate was occasionally applied dissolved in 1% (w/v) agarose in the appropriate phosphate/citrate buffer. A more permanent staining for β-galactosidase was obtained with a solution of 3 mg of 6-bromo-2-naphthyl β-D-galactoside in 0.5 ml of dimethyl sulphoxide and 9.5 ml of appropriate 1976
buffer, to which was added 10mg of Garnet GBC salt (G. T. Gurr, London S.W.6, U.K.). The substrate was applied on Whatman no. 4 filter paper and the gel wrapped in polythene sheeting and incubated overnight.

ISOELECTRIC FOCUSING

An LKB 8100 isoelectric-focusing column (capacity 110 ml) was used as described by Winchester (1971) by using Ampholines giving a pH range of 3–6. Enzyme preparations (2–3 ml) were placed in the two central fractions. Focusing was allowed to proceed for 48h, by which time the haemoglobin had formed itself into very sharp bands and some proteins had precipitated. The column contents were run out at 60ml/h and collected in 2.0ml fractions.

RESULTS

Most of the following investigations have been carried out on human liver samples that had been stored for several months at −20°C. Liver homogenates (20%, w/v, in water) were prepared with a top-drive homogenizer (MSE Instruments, Crawley, Sussex, U.K.). The β-galactosidase activity of these homogenates was 4.50±0.82nmol/min per mg of protein (11 samples), assayed at pH4.35. Specific activities of more recently frozen samples were within this range. β-Glucosidase activity assayed at pH6.0 was normally 10–20% of the β-galactosidase activity assayed at pH4.35. The homogenates were then centrifuged at 37500g for 30min at 4°C in an MSE 18 centrifuge with an angle-head rotor (11cm). The sedimented material, when dispersed in water and assayed, contained 10% of the total activity of the homogenates. Sedimented activity, solubilized by shaking with 0.1% Triton X-100 for 2min, was indistinguishable from the non-sedimented activity on gel filtration, electrophoresis or ion-exchange chromatography, and contained the same isoenzymic forms in about the same proportions.

GEL FILTRATION

The elution pattern of β-galactosidase activity from a relatively small column of Sephadex G-150 (45cm×2.5cm diam.) was similar to that previously described for human liver (Hultberg & Ockerman, 1969; Ho & O'Brien, 1971; Norden et al., 1974). Overall recoveries of 80–90% were obtained on elution with 10mm-sodium phosphate buffer, pH6.0, containing 5mm-NaCl. There was an excluded fraction, called β-galactosidase A because of its acidic pH optimum and lack of activity towards methylumbelliferyl β-D-glucoside, followed by a peak of the same type with an apparent molecular weight of 75000–85000. Finally came a peak named β-galactosidase B because of its higher pH optimum and ability to hydrolyse both the β-galactoside and the β-glucoside substrate. The apparent molecular weight of this activity was 40000–45000; the ratio of its activities towards methylumbelliferyl glucoside and methylumbelliferyl galactoside was 2:1 and its total activity varied widely between different liver samples. In preliminary experiments the ratio of activities of the two β-galactosidase A peaks appeared to vary with the ionic strength of the eluting buffer. To test this, samples of the same liver extract were incubated overnight at 4°C in 10mm-sodium phosphate buffer, pH7.0, containing NaCl at concentrations ranging from 5mm to 1.0m, before application to the column and elution with the same buffer. The total amount of β-galactosidase B activity did not vary, but the activity of the excluded peak increased with increasing concentration of NaCl, whereas that of the middle peak fell. Under the conditions used, the ratio of activities of high-molecular-weight β-galactosidase A to the lower-molecular-weight A form varied from 2.3:1 to 5.4:1, suggesting that a salt-dependent aggregation had occurred.

When the liver supernatants were filtered through a large column of Sephadex G-150 (100cm×2.5cm diam., exclusion volume 225ml), eluted with 10mm-sodium phosphate buffer containing 5mm-NaCl at the lower flow rate of 21ml/h, an increased resolution of β-galactosidase A activity was obtained (Fig. 1). A new intermediate form (β-galactosidase A2) with an apparent molecular weight of 160000–170000 appeared between the excluded activity (A3) and the previously described β-galactosidase A1 peak of 80000 molecular weight.

Fig. 1. Gel filtration of human liver β-galactosidase

Conditions of chromatography are detailed in the Methods section. Eluent fractions were assayed for β-galactosidase activity at pH4.35 (●) and β-glucosidase at pH6.0 (○), as described in the Methods section.
Conditions of chromatography are described in the Methods section. Fractions were assayed for $\beta$-galactosidase at pH 4.35 (○) and $\beta$-glucosidase at pH 6.0 (○) as described in the Methods section. The salt gradient was measured conductimetrically (-----).

The ability of all three $\beta$-galactosidase A peaks to hydrolyse GM$_1$ ganglioside was assayed and each showed the equal specific activities towards GM$_1$ ganglioside and methylumbelliferyl galactoside previously reported for 'acid' $\beta$-galactosides (Ho et al., 1973).

**Ion-exchange chromatography**

The $\beta$-galactosidase activity of supernatants from human liver homogenates was completely retained by DEAE-cellulose equilibrated with 0.01M-sodium phosphate buffer, pH 6.0, and could be eluted with close to 100% recovery by the application of a linear concentration gradient of NaCl (Fig. 2). The first two peaks of activity that appeared coincided with peaks of methylumbelliferyl $\beta$-glucosidase activity, both activities being optimal at pH 6.0. The two peaks were pooled separately, concentrated by vacuum dialysis against 10mM-sodium phosphate buffer, pH 7.0, to specific activities of 0.5 and 2.2 nmol of methylumbelliferone/min per mg respectively, and filtered through a Sephadex G-150 column as described above. Both were eluted as single symmetrical peaks with the apparent molecular weight of $\beta$-galactosidase B. They were therefore named $\beta$-galactosidases B1 and B2 in order of their elution from DEAE-cellulose. After dialysis and concentration they were found to differ in their response to the presence of NaCl. The B1 activities were stimulated to a maximum of twofold at 10mM-NaCl, whereas the B2 activities were 80% inhibited at this concentration. No difference was found in the pH or thermal stabilities or in the substrate specificities of the two B forms. Each hydrolysed methylumbelliferyl $\beta$-D-glucoside, $\beta$-D-xylidoside and $\alpha$-L-arabinoside with the same relative activities. Both the B1 and B2 isoenzymes appeared inactive towards GM$_1$ ganglioside, even when the incubation time was extended to 1h.

In the further analysis of the major peak of activity (Fig. 2), fractions from the leading edge, the centre and the trailing edge were pooled separately and concentrated by vacuum dialysis. By determination of pH optima, and the lack of activity towards methylumbelliferyl $\beta$-glucoside, each concentrate was shown to consist of $\beta$-galactosidase A activity. The concentrates were applied separately to the larger Sephadex G-150 column and eluted with 10mM-sodium phosphate buffer, pH 7.0, containing 5 mM-NaCl. Each fraction contained all three A-type isoenzymes, but in varying proportions. In the early fractions isoenzymes A1 and A2 predominated, whereas isoenzyme A3 was greater in the trailing fractions.

**Starch-gel electrophoresis**

On starch-gel electrophoresis in 40mM-sodium phosphate buffer, pH 7.0, the $\beta$-galactosidase activity of human liver homogenates separated into three zones, previously labelled A, B and C in decreasing order of mobility (Ho & O'Brien, 1971). The single $\beta$-glucosidase zone coincided with the 'A' band. Greater resolution was obtained by applying the methylumbelliferyl substrate in 1% (w/v) agarose gel. The A band was found to contain two components of slightly different mobility, only the faster component displaying $\beta$-glucosidase activity. The separated peaks of A3, A2, A1 and B activity obtained from Sephadex G-150 filtration were concentrated and applied to starch gel (Plate 1a). On electrophoresis none of these activities migrated in the position of 'band C'. Only $\beta$-galactosidase A3 migrated as 'band B'; the remaining forms all migrated in the 'band A' position. The A1 and A2 forms were slightly slower than the B form, but the difference was not sufficient for them to be resolved by the usual electrophoretic technique when they were together in the same solution. Resolution of the components of the 'A band' was obtained by starch gel electrophoresis in two dimensions. The first separation was carried out in 40mM-sodium phosphate buffer, pH 7.0, and a 5mm-wide strip, long enough to include all the separated proteins, was cut from the centre of the band. The strip of gel was inserted at the origin line of a second starch gel made up in 40mM-sodium phosphate buffer, pH 5.0, and electrophoresis carried out in a direction perpendicular to the first (Plate 1b). The $\beta$-galactosidase A1 and A2 components of band A showed very little mobility in the second dimension, whereas $\beta$-galactosidase B moved rapidly towards the anode. The $\beta$-galactosidase A3 form did not move from its position in the sample gel strip.
Starch-gel electrophoresis of β-galactosidase isoenzymes separated by (a) Sephadex G-150 gel filtration and (c) DEAE-cellulose ion-exchange chromatography, and (b) two-dimensional starch-gel electrophoresis of β-galactosidase

(a) Samples of each isoenzyme were produced by concentrating peaks of activity eluted from the column by vacuum dialysis against 0.025M-sodium phosphate buffer, pH 7.0, containing 10mM-NaCl. Electrophoresis and detection of the enzyme are as described in the Methods section. (1) Human liver β-galactosidase homogenate; (2) β-galactosidase A3; (3) β-galactosidase A2; (4) β-galactosidase A1; (5) β-galactosidase B. (b) For the two-dimensional electrophoresis, human liver homogenate was electrophoresed in the first dimension in sodium phosphate buffer, pH 7.0, as illustrated in (a), sample (1). The strip of starch gel containing the partially separated β-glucosidase isoenzymes was cut out and re-embedded in a second gel at pH 5.0 and electrophoresed in the perpendicular direction in sodium phosphate buffer, pH 5.0. (c) β-Galactosidase isoenzymes were separated as shown in Fig. 2 and concentrated as in (a). (1) β-Galactosidase B1; (2) β-galactosidase B2; (3) a sample taken from the leading edge of the β-galactosidase A peak (predominantly β-galactosidases A1 and A2); (4) a sample taken from the trailing edge of the β-galactosidase A peak (predominantly β-galactosidase A3).
EXPLANATION OF PLATE 2

(a) Starch-gel electrophoresis of the conversion of $\beta$-galactosidase A3 into $\beta$-galactosidase A1 by repeated freezing and thawing and (b) effect of neuraminidase treatment on the electrophoretic mobilities of human liver $\beta$-galactosidase isoenzymes

Electrophoresis, enzyme staining and neuraminidase treatment were as described in the Methods section. In (a): (1) human liver homogenate; (2) $\beta$-galactosidase A3; (3) $\beta$-galactosidase A3 after 60 freeze–thaw cycles as described in the Results section. In (b): (1) neuraminidase-treated human liver supernatant; (2) control incubated with water instead of neuraminidase.

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Peaks of activities from DEAE-cellulose chromatography of human liver homogenates (Fig. 2) were also pooled, concentrated by vacuum dialysis and analysed by starch-gel electrophoresis (Plate 1c). Both β-galactosidase B1 and B2 migrated with the mobility of ‘band A’ and were indistinguishable. The β-galactosidase A activity was distributed between electrophoretic bands ‘A’ and ‘B’. Again no activity migrated in the position of ‘band C’. This band is only occasionally seen in human liver homogenates, and, in attempts to characterize it further, starch-gel electrophoresis was carried out on a larger scale by applying 0.1ml of supernatant (10gml) and the supernatant was filtered through a Sephadex G-150 column. The β-galactosidase activity was of the A-type, with the apparent molecular weight of β-galactosidase A1.

Isoelectric focusing

Isoelectric focusing of 2ml samples of supernatants (37500g for 30min) from human liver homogenates (40%, w/v) was carried out in sucrose-stabilized pH gradients with Ampholines of pH range 3-6. The column contents were run out after 48h and assayed for β-galactosidase at pH4.5 and 6.0 and for β-glucosidase at pH6.0. Three peaks of activity were obtained, having isoelectric points at pH4.0, 4.35 and 4.95 respectively (Fig. 3). Only the first of these had both β-glucosidase and β-galactosidase activity and therefore contained β-galactosidase B. The central fractions of each peak were pooled and dialysed against a 1% (w/v) glycine solution for analysis by starch-gel electrophoresis. The activity of the pH4.0 peak migrated as a broad single zone, as expected for a mixture of galactosidases A1, A2 and B. The activity of the middle peak migrated slightly more slowly, with the mobility of the A1 and A2 forms. The bulk of the activity of the third peak migrated in the position of β-galactosidase A3, although there was a minor zone of activity in the position of isoenzymes A1 and A2. Samples from each peak were also re-focused over the same pH range. In each case multiple peaks were obtained (varying between two and four) and there was a loss of up to 85% of the original activity.

Pooled and concentrated peaks of β-galactosidase A1, A2 and A3 activities from Sephadex G-150 separations were also subjected to the isoelectric-focusing procedure and each gave single symmetric peaks with apparent isoelectric points at pH4.53, 4.85 and 4.80 respectively. The B1 and B2 activities, separated by DEAE-cellulose chromatography, gave apparent isoelectric points of pH4.50 and 4.30. The isoelectric points obtained with the partially purified samples thus differed markedly from those that would be deduced from an analysis of a crude homogenate.

Interconversion of isoenzymic forms of β-galactoside

The experiment described above in which supernatants from human liver homogenates were filtered through Sephadex G-150 in buffers containing different NaCl concentrations suggested that the three β-galactosidase A forms were interconvertible, but that β-galactosidases B were independent of these. When the peaks of activities from such gel filtrations were pooled, concentrated separately and refiltered through the 100cm×2.5cm column of Sephadex G-150 as described above, it was found that the A2, A1 and B forms were each eluted as single symmetrical peaks and with unchanged elution volumes. The A3 form, on the other hand, gave rise to two additional peaks on refilteration, accounting for 20% of the total activity and corresponding in elution volume to the A1 and A2 forms. Incubation of the sample with 0.1% (w/v) Triton X-100 and the addition of 0.1% Triton X-100 to the elution buffer increased the activity in these peaks to 50% of the total. The proportion of the separated A3 form that was converted into form A1 or A2 could also be increased by freezing in an acetone/solid CO₂ mixture and thawing in a 37°C water bath. After 60 cycles the interconversion appeared complete, as shown by starch-gel-electrophoretic analysis (Plate 2a), and the activity was 110% of the original activity of β-galactosidase A3. Freezing and thawing had no
Effect on the gel-filtration or electrophoretic behaviour of the A1, A2 or B fractions. Other treatments that achieved a partial conversion of form A3 into a mixture of forms A1 and A2 were incubation with 0.1% sodium taurocholate for 6h in 0.01m-sodium phosphate buffer, pH7.0, and treatment with aqueous solutions (10%, v/v) of organic solvents. Methanol, ethanol and isopropyl alcohol had some effect, but the most successful was n-propyl alcohol, which produced 30% conversion. Sonication at a frequency of 20kHz and amplitude of 5µm for 4min also generated the A1 and A2 forms from form A3.

The variety of procedures that lead to interconversion of A forms raised the possibility that form A3 consisted of A activity bound to small fragments of lysosomal membrane. When the form-A3 peak from Sephadex G-150 filtration was pooled and concentrated overnight by vacuum dialysis and then centrifuged at 26000g for 30min, most of the activity was recovered in the pellet. Part of the pellet was resuspended in 40mM-sodium phosphate buffer and subjected to starch-gel electrophoresis to show that the β-galactosidase activity still migrated in the 'B band' characteristic of the A3 form. The remainder of the pellet was fixed in glutaraldehyde and OsO₄, and stained with uranyl acetate and lead acetate for examination under the electron microscope. No clear evidence for the presence of membranes was obtained.

Effect of neuraminidase on human liver β-galactosidases

 Supernatants (5×10⁵g-min) from human liver homogenates were incubated with Clostridium perfringens neuraminidase (1 unit/ml of supernatant) for 1h at 37°C without loss of β-galactosidase activity. Comparison with untreated homogenates on starch-gel electrophoresis showed that the activity of the fastest zone was diminished, and a new zone appeared, intermediate between bands A and B in mobility (Plate 2b). The new zone had no β-glucosidase activity. Treatment with neuraminidase had therefore decreased the mobilities of the β-galactosidase A1 or A2 components and had not affected β-galactosidase B. A slight decrease in the mobility of the A3 zone was also seen in the neuraminidase-treated extracts. Control experiments in which the neuraminidase preparation was boiled before the incubation showed that the effects observed were not due to the presence of a heat-stable factor.

That neuraminidase caused a decrease in the negative charge of a portion of the β-galactosidase A activity was shown by the DEAE-cellulose chromatography of neuraminidase-treated liver supernatant. It was found that 40% of the β-galactosidase A was co-eluted with the β-galactosidase B isoenzymes (cf. Fig. 2).

Additional evidence came from the isoelectric focusing of neuraminidase-treated liver supernatant, which shows that the isoelectric points of the neuraminidase-treated B1 and B2 isoenzymes were increased by 0.05 and 0.25pH unit whereas the A3, A2 and A1 forms had their pI values increased by 0.5, 0.43 and 0.77 respectively.

Discussion

By exploiting the improved resolution in gel-filtration experiments obtained when the column length was increased and the rate of elution decreased, the number of forms of ‘acid’ β-galactosidase found in human liver homogenates has been increased to three. The two low-molecular-weight forms A1 and A2 correspond to the ‘A’ form purified by Norden et al. (1974), and the high-molecular-weight A3 form corresponds to their ‘B’ form. All three β-galactosidase A forms hydrolyse GM₁ gangloside and are simultaneously absent in cases of GM₁ gangliosidosis (Okada & O'Brien, 1969), whereas the neutral β-galactosidase activity is unaffected. This evidence that the A forms are closely related was reinforced by the experiments on their interconversion. Isolated β-galactosidase A₃, on refiltration through Sephadex G-150 or G-200, gave rise to the A1 and A2 forms and this process was enhanced by a variety of treatments, especially those which disrupt protein–lipid bonds. However, form A1 has not been successfully generated from form A2, and preparations of either form A1 or A2 could not be converted back into form A3, after isolation by gel filtration. The evidence of a salt-dependent aggregation of β-galactosidase A presented here contradicts that of Norden et al. (1974), possibly because they did not preincubate the homogenate in the appropriate salt concentration before application to the Sephadex column.

The present evidence from sodium dodecyl sulphate/polyacrylamide-gel electrophoresis indicates that β-galactosidase A₁ consists of a single chain of 80000 molecular weight (Norden et al., 1974), suggesting that form A₁ is a monomeric form and that form A₂ of molecular weight 160000–170000 is a dimer. Hultberg & Ockerman (1972) also obtained evidence of this dimeric form by modification of their gel-filtration conditions. As β-galactosidase A₃ is excluded from Sephadex G-200 it has not been possible to assign a molecular weight or to be sure that it consists of a single isoenzymic form. O'Brien (1975) has speculated that the high-molecular-weight ‘acid’ β-galactosidase is a multimeric form of the lower-molecular-weight form. An alternative explanation is that it consists of the low-molecular-weight form bound to small fragments of lysosomal membrane. Procedures that disrupt lipid–protein links are effective in generating forms A1 and A2.
from form A3, and only the A3 form is precipitated at pH 5.0 (Ho et al., 1973), behaviour characteristic of lipoproteins (Coleman, 1974). The possibility that the high-molecular-weight activity consists of more than one component cannot be excluded.

None of the procedures used was able to generate the ‘neutral’ β-galactosidase B forms from β-galactosidase A3, in agreement with the evidence from GM1 gangliosidosis that they are under separate genetic control. The suggestion (Ockerman, 1969) that the high-molecular-weight form contained a mixture of the low-molecular-weight ‘acid’ and ‘neutral’ forms might be explained by the presence of the specific ‘acid’ β-glucosidase in the material excluded from the Sephadex gel. The low pH used in the β-glucosidase assay would emphasize the presence of this enzyme, which has been found tightly bound to membranes in human liver lysosomes (Beck & Tappel, 1968). To distinguish it from the neutral β-glucosidase activity we suggest that it be named β-glucosidase A. It appears to be the enzyme that hydrolyses ceramide glucoside and is deficient in Gaucher’s disease (Brady et al., 1965).

No role has yet been found for the ‘neutral’ β-galactosidase, which is widely distributed in mammalian tissues and appears equally active in hydrolysing β-d-galactosides. It will also hydrolyse β-d-xyllo- and α-L-arabinosides, but at a much lower rate. By using DEAE-cellulose chromatography this activity has been separated into two fractions, B1 and B2, which were not separable by any of the other techniques applied, and which had apparently identical substrate specificities but differed in their response to Cl– ions. This activity shows an unusually wide range of specific activities in ‘normal’ tissue, especially after freezing, and is frequently found to be absent from pathological samples. This was the origin of some temporary confusion in the study of GM1 gangliosidosis and in particular in the diagnosis of the enzymic lesion (Pinsky et al., 1974).

It is clear from these results that analysis of human liver homogenates by gel electrophoresis should be viewed with caution. The fast electrophoretic band ‘A’ normally contains β-galactosidases A1, A2 and B, although these could be resolved by a two-dimensional electrophoretic technique (Plate 1). The band of intermediate mobility ‘B’ consists simply of β-galactosidase A3. The slow diffuse zone ‘C’ yielded only β-galactosidase A1 when the zone was cut out of the gel and the activity solubilized. Possibly it consists of β-galactosidase A1 or A2 (or both) bound to rather larger membrane fragments than for β-galactosidase A3. The presence of a ‘C’ band would then depend on the conditions of homogenization and this would explain why it is not invariably seen in liver homogenates. Treatment of the liver homogenates with neuraminidase caused a decrease in the mobilities of β-galactosidase A1 and A2, although that of β-galactoside B was unaffected. This probably reflects a genuine difference in sialic acid content between the A and B types of isoenzyme, as a similar change in mobility was not observed after treatment with an inactivated neuraminidase preparation.

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