Biochemical studies on a case of feline mannosidosis

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Evidence is presented for the biochemical diagnosis of the first case of feline mannosidosis. A marked deficiency of acidic α -D-mannosidase in the brain, kidney and liver and excessive excretion of mannose-rich oligosaccharides in the urine were found in a kitten suffering from a nervous disorder. Residual acidic α -D-mannosidase, ranging from 2 to 5.5% of the normal activity, was observed in the tissues of the affected kitten. It had similar kinetic and physicochemical properties to the normal activity. The amount of mannose in the urine of the affected kitten was 19-fold greater than in a comparable control, and the molar ratio of mannose to *N*-acetylglucosamine was approx. 6:1. High concentrations of neutral oligosaccharides were detected in the urine. The predominant oligosaccharide appeared to be a hexasaccharide. The biochemical features of bovine, feline and human mannosidosis are compared, and it is concluded that feline mannosidosis may be a useful animal model for studying the human disease.

Mannosidosis is the lysosomal storage disease resulting from a deficiency of acidic α -D-mannosidase (α -D-mannoside mannohydrolase, EC 3.2.1.24). It has been described in humans (Öckerman, 1967; Carroll *et al.*, 1972) and in Angus cattle (Whittem & Walker, 1957; Jolly, 1971; Hocking *et al.*, 1972). As a consequence of this deficiency, there is accumulation of mannose-rich material in the tissues (Kjellman *et al.*, 1969; Hocking *et al.*, 1972) and excretion in the urine of mannose-rich oligosaccharides (Nordén *et al.*, 1973a; Lundblad *et al.*, 1975). Diagnosis of the disease is based on demonstrating a deficiency of acidic α -D-mannosidase in tissues or body fluids and/or the detection of mannose-rich oligosaccharides in the urine, together with clinical symptoms. It has been concluded from characterization of the residual acidic α -D-mannosidase present in mannosidosis that mutations in the structural genes for lysosomal acidic α -D-mannosidase are responsible for human and bovine mannosidosis (Taylor *et al.*, 1975; Burditt *et al.*, 1978). In the present paper, biochemical studies on a case of mannosidosis in a cat are reported. Clinical and pathological studies have also been performed (W. F. Blakemore, unpublished work). The biochemical features of bovine, feline and human

mannosidosis have been compared because feline mannosidosis may provide a convenient animal model for the human disease.

Materials and methods

Tissues

Samples of the brain, kidney and liver of a 9-month-old female domestic kitten with nervous symptoms, physical deformity and retarded growth were stored at -20°C after euthanasia. Diagnosis of a storage disease was based on microscopical studies and vacuolation of lymphocytes (85%) (W. F. Blakemore, unpublished work). The corresponding organs of an age- and sex-matched control were also stored frozen. Tissue homogenates (10 or 20%, w/v) were prepared in a Potter–Elvehjem homogenizer in water or the appropriate buffer for a separation procedure. The supernatants obtained after centrifugation of the homogenates in the $8 \times 50\text{ml}$ angle rotor of an MSE High-Speed 18 centrifuge for 15 min at 4°C and $37000g$ (r_{av} , 107 mm) were used.

Assay for α -D-mannosidase

α -D-Mannosidase was assayed by using the fluorogenic substrate 4-methylumbelliferyl α -D-

mannopyranoside (Koch–Light Laboratories, Colnbrook, Bucks., U.K.) with a substrate concentration of 1 mM as described previously (Phillips *et al.*, 1974a). Activity measured at pH 4.0 and 6.5 was termed acidic and neutral α -D-mannosidase respectively. One unit of activity is the amount that transforms 1 μ mol of substrate/min under the specified conditions. The McIlvaine (1921) phosphate/citrate buffer system was used to investigate the pH-dependence of the α -mannosidase activity in the presence and absence of 1 mM- CoSO_4 and $-\text{ZnSO}_4$. The values of K_m were calculated by the graphical method of Lineweaver & Burk (1934), by using a computer program to obtain the line of best fit by the least-squares method for the regression line.

Other enzyme assays

Eight other lysosomal hydrolases were assayed by using the appropriate fluorogenic 4-methylumbelliferyl glycoside (Koch–Light). All the assays were performed in the phosphate/citrate buffer system at pH 4.0 with a substrate concentration of 0.5 mM, except for α -L-fucosidase, which was assayed at pH 5.25. A substrate concentration of 0.5 mM was also used to assay α -mannosidase in the initial screening of the tissues.

Thermal stability of α -mannosidase

Supernatants from tissue homogenates (20%, w/v) were heated for 1 h at various temperatures before being assayed for α -mannosidase at 37°C in the standard manner. The rate of denaturation of the acidic α -mannosidase in the control and affected cat kidney was measured at 60°C.

Chromatography on DEAE-cellulose and concanavalin A–Sephrose

Ion-exchange chromatography on DEAE-cellulose (Whatman DE-52) and affinity chromatography on concanavalin A–Sephrose [Pharmacia (G.B.), London W5, U.K.] were carried out as described previously (Phillips *et al.*, 1974a, 1976).

Analysis of urinary oligosaccharides

Samples (3 ml) of urine from the control and affected kittens were passed through a column of mixed-bed resin (Zerolit DMF; Hopkin and Williams, Romford, Essex, U.K.) to remove charged material. After freeze-drying, the samples were re-dissolved in water (1 ml) and analysed by the following procedures.

(1) *Determination of total carbohydrate.* The Winzler orcinol/ H_2SO_4 procedure (François *et al.*,

1962) was used to measure the total carbohydrate in 100 μ l of the samples. A standard curve for mannose was used over the range 0–500 μ g.

(2) *Gas–liquid chromatography.* Samples of the normal urine (333 μ l) and of the pathological urine (10 μ l) were freeze-dried after the addition of 1 μ l of a 1 mM aqueous solution of *meso*-inositol as an internal standard. Methanolic hydrogen chloride (1.0 ml, 0.5 M) was added to each sample in an ampoule, which was then sealed and heated at 80°C for 2 h (Zanetta *et al.*, 1972). The cooled solutions were neutralized by adding Ag_2CO_3 (Clamp *et al.*, 1971). The methanolysed monosaccharides were dried under N_2 and the residue was redissolved in 0.5 ml of anhydrous methanol. To this was added 10 μ l of redistilled pyridine, followed by 50 μ l of acetic anhydride (Kozulic *et al.*, 1979). The reaction mixture was left at room temperature for 15 min and dried under N_2 . The samples were trimethylsilylated by the addition of 0.1 ml of a mixture of trimethylchlorosilane, hexamethyldisilazane and pyridine (1:3:9, by vol.) (Sigma Sil-A) for up to 30 min at room temperature. After drying under N_2 , the final derivatives were redissolved in 10 μ l of dry redistilled heptane. Samples (1 μ l) were analysed in a Pye Unicam 204 gas–liquid chromatograph with a flame ionization detector. Glass columns (150 cm long, 6 mm and 4 mm external and internal diam. respectively) containing 3% silicone SE-30 on Diatomite CLQ (mesh 100–120; J.J.'s Chromatography Ltd., King's Lynn, Norfolk, U.K.) were used with N_2 as the carrier gas at 30 ml/min and a temperature program from 130 to 250°C.

(3) *G.l.c.–mass spectrometry.* Silylated samples (1 μ l) of the urines prepared as described above were also analysed by coupled g.l.c.–mass spectrometry by using a Perkin Elmer Sigma 3 gas–liquid chromatograph and a Kratos MS 25 mass spectrometer coupled to a Kratos DS 50 Data System (Kratos Ltd., Manchester, U.K.). A glass column (270 cm $\times$ 4 mm internal diam.) packed with 3% silicone SE-30 on Diatomite CLQ (100–120 mesh) was used, with a temperature program from 130 to 250°C and He as the carrier gas at 30 ml/min for g.l.c. The 70 eV fragmentation patterns of the samples were recorded every 4 s throughout the run. An ion-source temperature of 200°C, an ionizing current of 100 μ A and an accelerating voltage of 1.3 kV were used. The presence of pertrimethylsilylated methyl glycosides was identified by their characteristic ions (Kamerling *et al.*, 1975).

(4) *Thin-layer chromatography.* Samples (10–15 μ l) were analysed on silica-gel plates (Merck 7515) with butan-1-ol/acetic acid/water (10:5:2, by vol.) as the solvent (Humbel & Collart, 1975). Neutral oligosaccharides were detected with orcinol [0.2% (w/v) in H_2SO_4 (20%, w/v, in water)]. Comparable samples of normal human urine, urine

from a patient with mannosidosis and from a calf with mannosidosis were also analysed.

Results

Lysosomal enzyme activities

Nine lysosomal enzymic activities were measured in the brain, kidney and liver of the cat under investigation and in the corresponding tissues of an age- and sex-matched control (Table 1). There was a marked deficiency of acidic α -D-mannosidase in all three tissues from the affected animal compared with the activity in the control. The proportions of residual acidic α -D-mannosidase were 2.8% in the brain, 5.5% in the kidney and 1.9% in the liver. The β -D-glucosidase activity in the affected cat was also lower than in the control, but the proportion of residual activity was in the range 60–85%. The activities of all other lysosomal enzymes were greater by severalfold in the tissues of the affected cat than in the control. It is concluded that there is a deficiency of lysosomal acidic α -D-mannosidase and secondary hyperactivity of the other enzymes except β -D-glucosidase in this animal. To investigate whether only the lysosomal form of α -D-mannosidase was deficient, the pH-dependence and the multiple forms of α -D-mannosidase in the two animals were compared.

pH-Dependence of the α -D-mannosidase

The activity–pH profiles for the α -D-mannosidase activity in the kidney and liver of the normal cat have two pH optima, one at pH 4.0–4.25 and the other at pH 6.5 (Fig. 1). The activity in the normal brain also has a pH optimum at pH 4.25 and a marked shoulder between pH 6.0 and 7.0. These profiles indicate that the acidic α -D-mannosidase with a pH optimum near pH 4.0 and neutral α -D-mannosidase with a pH optimum of 6.5 are both present in normal cat tissues. Acidic α -D-mannosidase was almost absent from the tissues of the affected cat, whereas the neutral α -D-mannosidase was present in a comparable amount in the brain and in decreased amounts in the kidney and liver.

Chromatography on DEAE-cellulose

Chromatography of an extract of the control liver on DEAE-cellulose showed that most of the acidic α -D-mannosidase bound to the ion-exchanger and that the neutral α -D-mannosidase was associated with a discrete, bound peak of activity (Fig. 2a). By analogy with the multiple forms of the enzyme in other mammalian tissues (Carroll *et al.*, 1972; Phillips *et al.*, 1974a) the peaks of unbound and bound acidic α -D-mannosidase were called A and B respectively, and the neutral α -D-mannosidase was called peak C. Fig. 2(b) shows that both acidic

Table 1. Glycosidase activities in the tissues of the diseased kitten and control
Enzymic activities were measured in supernatants from tissue homogenates (20%, w/v) at pH 4.0 (except for α -L-fucosidase, pH 5.25) by using the appropriate fluorogenic substrate.

Tissue	Enzyme activity (munits/mg of protein)									
	α -D-Glucosidase	β -D-Glucosidase	α -D-Galactosidase	β -D-Galactosidase	Fucosidase	α -L-Mannosidase	α -D-Mannosidase	β -N-Acetyl-D-glucosaminidase	β -N-Acetyl-D-galactosaminidase	β -D-Glucuronidase
Brain										
Control	0.18	0.07	0.42	0.95	1.42	1.42	2.68	7.86	2.53	1.10
Mannosidosis	0.73	0.044	7.05	19.50	8.25	8.25	0.074	101.8	36.4	30.2
Kidney										
Control	0.40	3.39	4.62	3.03	6.89	6.89	7.44	52.59	11.59	1.75
Mannosidosis	1.52	1.96	37.1	21.6	78.6	78.6	0.41	301.8	79.2	26.2
Liver										
Control	0.25	0.55	1.55	0.93	4.86	4.86	1.96	31.87	10.29	0.93
Mannosidosis	1.10	0.46	7.88	5.81	82.70	82.70	0.038	75.30	28.62	3.67

Fig. 1. pH-dependence of α -D-mannosidase in tissues of control and affected kittens

Supernatants from 20% (w/v) homogenates were assayed at a series of pH values with a substrate concentration of 0.5 mM. ●, Control; ○, affected kitten.

α -D-mannosidases A and B were virtually absent from the liver of the affected cat, but that α -D-mannosidase C was present. It also shows that the activity at pH 6.5 in peaks A and B in the normal tissues was due to acidic α -mannosidase. These results suggest that only the acidic α -D-mannosidase components are affected in this disorder.

Fig. 2. Chromatography on DEAE-cellulose of α -D-mannosidase in livers of (a) control and (b) affected kittens

Supernatants (1 ml) from 20% (w/v) homogenate were applied to a column (2 ml) of DEAE-cellulose. Fractions (2.2 ml) were collected at 60 ml/h. A NaCl gradient (0–0.3 M; ----) was used to elute bound protein. ●, α -D-Mannosidase assayed at pH 4.0; ○, α -D-Mannosidase at pH 6.5. Peaks A, B and C are defined and discussed in the text.

Partial characterization of the residual acidic α -D-mannosidase

Some properties of the acidic α -D-mannosidase activity in the tissues of the affected cat and of the control were compared to investigate whether a mutant enzyme was responsible for the small proportion of residual activity in the diseased animal.

(a) *Effect of Co^{2+} and Zn^{2+} .* Both the normal feline acidic α -D-mannosidase and the residual acidic α -D-mannosidase in the liver of the affected cat were activated by 1 mM- Zn^{2+} at the optimal pH (Fig. 3). There was also stimulation of the activity in both samples in the range pH 4.5–5.0 and slight inhibition of the neutral activity above pH 6.5. In contrast, Co^{2+} inhibited the acidic activity in both samples at pH values below 4.5 and stimulated the activity at pH values above 4.5. These observations suggest that the residual acidic α -D-mannosidase in the liver of the affected cat has the same response to Co^{2+} and Zn^{2+} ions as the normal enzyme. Feline neutral α -D-mannosidase appears to have similar properties to other mammalian forms of the enzyme (Phillips *et al.*, 1974a,b). The presence of α -D-

Fig. 3. Effect of Co^{2+} and Zn^{2+} on α -D-mannosidase in livers of (a) control and (b) affected kittens

The α -D-mannosidase activity in the livers of the control and affected kitten was assayed at a series of pH values in the presence and absence of 1 mM- Co^{2+} and Zn^{2+} . ●, No ions; ○, Co^{2+} ; ■, Zn^{2+} .

mannosidase activity that is activated by Co^{2+} and Zn^{2+} in the range pH 4.5–5.5 has been interpreted as the presence of intermediate α -D-mannosidase in human and bovine tissues (Phillips *et al.*, 1974b; Burditt *et al.*, 1978). It is possible therefore that this form of the enzyme is also present in the livers of the normal and affected cats.

(b) *Effect of substrate concentration on activity.* The values of K_m for the activity in the normal and pathological organs respectively were 1.4 and 1.4 mM in the brain, 1.43 ± 0.5 ($n=4$) and 3.06 ± 2.73 ($n=3$) mM for the kidney and 1.09 ± 0.38 ($n=3$) and 0.79 ± 0.13 ($n=3$) mM for

Fig. 4. T.l.c. of urinary oligosaccharides

T.l.c. of samples of urine and standards was carried out on silica gel with butan-1-ol/acetic acid/water (10:5:2, by vol.) as the solvent. Oligosaccharides were detected with orcinol/ H_2SO_4 . 1, Raffinose; 2, normal human urine; 3, human urine, mannosidosis; 4, bovine urine, mannosidosis; 5, feline urine, mannosidosis; 6, normal feline urine; 7, lactose.

the liver. Although the K_m value for the activity in the affected kidney varied, generally the K_m values in the normal and corresponding affected tissues were similar.

(c) *Chromatography on concanavalin A-Sepharose.* The acidic α -D-mannosidase in both the normal and affected liver bound to concanavalin A-Sepharose. This suggests that the enzyme responsible for the residual activity is also a glycoprotein. The neutral α -D-mannosidase in both samples did not bind to concanavalin A.

(d) *Thermal stability.* Incubation of extracts of the normal and affected tissues at a series of temperatures showed that the normal and residual acidic α -D-mannosidase activities were stable up to 50°C, but could be denatured at 60°C. When extracts of the kidneys were heated at 60°C for 1 h, the loss of activity was a first-order process and the two activities had a similar first-order rate constant. It was concluded that the normal and residual activities had a similar thermal stability.

Analysis of urinary oligosaccharides

The concentrations of the total neutral carbohydrate in the urines as measured by the orcinol/ H_2SO_4 reagent were 0.33 and 2.33 mg/ml of urine for the normal and pathological urines respectively. The nature of the carbohydrate material excreted by the two animals was investigated by g.l.c. The predominant monosaccharide present in the hydrolysate of the material from the diseased kitten was mannose. Lower concentrations of glucosamine and glucose were also present. The molar ratio of mannose to glucosamine was approx. 6:1. Mannose and glucose, but not hexosamine, were detected in

the control urine. The amount of mannose in the pathological sample was 19-fold greater than in the control sample when the results were calculated relative to the same weight of total carbohydrate in the two samples. Confirmation of the identity of the monosaccharides in the hydrolysates of the material in the urines of the kittens was obtained by g.l.c./mass spectrometry. All the carbohydrate-containing peaks in the g.l.c. elution profile were identified, and there were no unassigned fragments in the mass spectrogram.

Analysis of the urines by t.l.c. showed that there was a high concentration of neutral oligosaccharides in the urine of the diseased kitten, but not in the control (Fig. 4). The predominant component appeared to be a hexa- or possibly a penta-saccharide. The mixture of oligosaccharides excreted by the diseased cat was different from those in the urines of cases of bovine and human mannosidosis.

Discussion

Diagnosis of bovine or human mannosidosis is based on demonstrating a deficiency of acidic α -D-mannosidase in the tissues or body fluids and the detection of mannose-rich oligosaccharides in the urine together with clinical symptoms. The deficiency of acidic α -D-mannosidase in the brain, kidney and liver, the excessive excretion of mannose-rich oligosaccharides in the urine and the extensive vacuolation of lymphocytes, visceral organs and cells of the nervous system of the kitten studied (W. F. Blakemore, unpublished work) justify diagnosis of the first case of feline mannosidosis.

The enzymic defect affects both forms of acidic α -D-mannosidase, A and B, separable by chromatography on DEAE-cellulose, but not the neutral α -D-mannosidase, peak C. A similar situation occurs in human (Carroll *et al.*, 1972) and bovine mannosidosis (Phillips *et al.*, 1974a). There was a small amount of residual acidic α -D-mannosidase in the tissues of the kitten with mannosidosis, the highest proportion and concentration being in the kidney. Preliminary characterization of this residual activity showed that it had similar kinetic and physicochemical properties to the normal enzyme. In contrast, the mutant enzymes responsible for the residual activity in bovine and human mannosidosis differ in several respects from the corresponding normal enzymes. Whereas the residual activity in feline mannosidosis had a thermostability comparable with that of the normal enzyme, the mutant enzymes in the other two species are markedly less stable than the activities in normal tissues (Beaudet & Nichols, 1976; Burditt *et al.*, 1978). Similarly, in bovine mannosidosis (Burditt *et al.*, 1978) and in most but not all cases of human mannosidosis the

K_m value for the mutant enzyme is greater than for the normal enzyme. In feline mannosidosis the activity in an affected tissue generally had similar kinetics to the activity in the corresponding normal tissue, although the K_m value for the activity in the kidney of the cat with mannosidosis varied from one determination to another. The normal and residual acidic α -D-mannosidase in bovine and feline tissues is inhibited by Co^{2+} (Phillips *et al.*, 1974a). However, the mutant enzyme in human mannosidosis is activated by Co^{2+} (Masson *et al.*, 1974; Burditt *et al.*, 1980). The affinity of the enzyme for concanavalin A is unaltered in the disease in all three species (Burditt *et al.*, 1978, 1980). A mutation in the structural gene for acidic α -D-mannosidase has been postulated in bovine (Burditt *et al.*, 1978) and human mannosidosis (Taylor *et al.*, 1975). If there is a mutation in the structural gene in feline mannosidosis, it does not appear to affect the properties of the enzyme investigated by us. However, without purification and further characterization of the residual activity it is not possible to conclude that there is a decreased concentration of the normal enzyme, resulting from a defect in a regulatory gene. A structural mutation could lead to an enhanced susceptibility to proteolysis or an alteration in another property that results in a decreased activity in the tissues. Further, the normal and residual enzymes may have different activities on natural substrates.

There is very little information about the normal excretion of neutral oligosaccharides in feline urine, but a combination of g.l.c. and mass spectrometry showed that there was a much higher excretion (19-fold) of mannose-containing material in the affected kitten than in the control. Comparable increases in the excretion of mannose have been observed in bovine (Nordén *et al.*, 1973b) and human mannosidosis (Nordén *et al.*, 1973a). T.l.c. of the urines showed that there were much higher concentrations of neutral oligosaccharides in the urine in mannosidosis than in the normal urine. The pattern of excreted oligosaccharides in feline mannosidosis was different from those in bovine and human mannosidosis, in which a penta- and a tri-saccharide respectively are predominant (Nordén *et al.*, 1973a; Lundblad *et al.*, 1975). Comparison of the chromatograms for the three species suggests that the predominant oligosaccharide in feline mannosidosis is a hexasaccharide, but a tetra-saccharide and higher oligomers are also present. A hexasaccharide containing five mannose and one *N*-acetylglucosamine residues has been isolated as a minor component from the urine of a patient with mannosidosis (Strecker *et al.*, 1976). Its structure is consistent with the incomplete catabolism of the poly-mannose type of carbohydrate side chain in glycoproteins. Mannose and hexosamine in the

molar ratio of approx. 6:1 were the major sugars detected by g.l.c./mass spectrometry in hydrolysates of the feline-mannosidosis urine. Therefore it is probable that the feline oligosaccharides consist of incompletely catabolized fragments of *N*-glycosidally linked carbohydrate side chains of glycoproteins. The high ratio of mannose to *N*-acetylglucosamine and the size of the predominant oligosaccharide might further suggest that the poly-mannose type of side chain is prevalent in feline glycoproteins. Alternatively the specificity of the residual activity rather than the structure of the natural substrates may account for the nature of the storage products.

Biochemically, feline mannosidosis shows similarities to and differences from both bovine and human mannosidosis. Clinically, the extent and the appearance of the cells more closely resemble those in the severe human variant (Sung *et al.*, 1977) than in the bovine disease (Jolly & Thompson, 1977). Thus feline mannosidosis may be a further useful animal model for studying the human disease.

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References

- Beaudet, A. L. & Nichols, B. L. (1976) *Biochem. Biophys. Res. Commun.* **68**, 292–298
- Burditt, L. J., Phillips, N. C., Robinson, D., Winchester, B. G., Van-De-Water, N. S. & Jolly, R. D. (1978) *Biochem. J.* **175**, 1013–1022
- Burditt, L., Chotai, K., Halley, D. & Winchester, B. (1980) *Clin. Chim. Acta* in the press
- Carroll, M., Dance, N., Masson, P. K., Robinson, D. & Winchester, B. G. (1972) *Biochem. Biophys. Res. Commun.* **49**, 579–583
- Clamp, J. R., Bhatti, T. & Chambers, R. E. (1971) *Methods Biochem. Anal.* **19**, 229–344
- François, C., Marshall, R. D. & Neuberger, A. (1962) *Biochem. J.* **83**, 335–340
- Hocking, J. D., Jolly, R. D. & Batt, R. D. (1972) *Biochem. J.* **128**, 69–78
- Humbel, R. & Collart, M. (1975) *Clin. Chim. Acta* **60**, 143–145
- Jolly, R. D. (1971) *J. Pathol.* **103**, 113–121
- Jolly, R. D. & Thompson, K. G. (1977) *J. Pathol.* **121**, 59–62
- Kamerling, J. P., Gerwig, G. J., Vliegthart, J. F. G. & Clamp, J. R. (1975) *Biochem. J.* **151**, 491–495
- Kjellman, B., Gamstorp, I., Brun, A., Öckerman, P. & Palmgran, B. (1969) *J. Pediatr.* **75**, 366–373
- Kozulic, B., Ries, B. & Mildner, P. (1979) *Anal. Biochem.* **94**, 36–39
- Lineweaver, H. & Burk, D. (1934) *J. Am. Chem. Soc.* **56**, 658–666
- Lundblad, A., Nilsson, B., Nordén, N. E., Svensson, S., Öckerman, P. A. & Jolly, R. D. (1975) *Eur. J. Biochem.* **59**, 601–606
- Masson, P. K., Lundblad, A. & Autio, S. (1974) *Biochem. Biophys. Res. Commun.* **56**, 296–303
- McIlvaine, T. C. (1921) *J. Biol. Chem.* **49**, 183–186
- Nordén, N. E., Lundblad, A., Svensson, S., Öckerman, P. A. & Autio, S. (1973a) *J. Biol. Chem.* **248**, 6210–6215
- Nordén, N. E., Lundblad, A., Öckerman, P. A. & Jolly, R. D. (1973b) *FEBS Lett.* **35**, 209–212
- Öckerman, P. A. (1967) *Lancet* **ii**, 239–241
- Phillips, N. C., Robinson, D., Winchester, B. G. & Jolly, R. D. (1974a) *Biochem. J.* **137**, 363–371
- Phillips, N. C., Robinson, D. & Winchester, B. G. (1974b) *Clin. Chim. Acta* **55**, 11–19
- Phillips, N. C., Robinson, D. & Winchester, B. G. (1976) *Biochem. J.* **153**, 579–587
- Strecker, G., Fournet, B., Bouquelet, S., Montreuil, J., Dhondt, J. L. & Farriaux, J. P. (1976) *Biochimie* **58**, 579–586
- Sung, J. H., Hayand, M. & Desnick, R. J. (1977) *J. Neuropathol. Exp. Neurol.* **36**, 807–820
- Taylor, H. A., Thomas, G. H., Aylsworth, A., Stevenson, R. E. & Reynolds, L. W. (1975) *Clin. Chim. Acta* **59**, 93–99
- Whittem, J. H. & Walker, D. (1957) *J. Pathol. Bacteriol.* **74**, 281–288
- Zanetta, J. P., Breckenridge, W. C. & Vincendon, G. (1972) *J. Chromatogr.* **69**, 291–301