A Serological Investigation into the Acidic α -D-Mannosidase in Normal Angus Cattle and in a Calf with Mannosidosis

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Antiserum was raised in a rabbit against bovine kidney acidic α -mannosidase that had been purified 570-fold by affinity chromatography on concanavalin A-Sepharose and Sepharose 4B- ϵ -aminohehexanoylmannosylamine. The antiserum precipitated the acidic but not the neutral α -mannosidase in normal calf tissues. Human acidic α -mannosidase cross-reacted partially with the antiserum, emphasizing the close structural resemblance between the enzyme in the two species. The residual acidic α -mannosidase in the tissues of a calf with mannosidosis was also precipitated by the antiserum, the same volume of antiserum being required to precipitate a unit of α -mannosidase activity from the normal and pathological tissues. The concentration of cross-reacting material detected by antibody-consumption experiments in the organs of the calf with mannosidosis appeared to be proportional to the concentration of the residual acidic α -mannosidase. It is suggested that the residual acidic α -mannosidase in mannosidosis accounts for the cross-reacting material detected and that it is unlikely that enzymically inactive but cross-reacting material is present. The residual acidic α -mannosidase could be a decreased concentration of the normal gene product or an altered enzyme with a decreased specific enzymic activity and a correspondingly decreased antigenicity.

Bovine mannosidosis is an inherited, lethal, lysosomal storage disease of Angus cattle (Whittem & Walker, 1957; Jolly, 1971) resulting from a deficiency of lysosomal acidic α -D-mannosidase (EC 3.2.1.24) (Hocking *et al.*, 1972; Phillips *et al.*, 1974a). The clinical and pathological aspects of the disease, its biochemical basis and mode of inheritance, the detection of heterozygotes with regard to a screening programme and its potential as an animal model for human lysosomal storage diseases have been reviewed (Jolly, 1975). A very similar disease occurs in humans (Öckerman, 1967; Kjellman *et al.*, 1969; Carroll *et al.*, 1972) and the development of reliable biochemical diagnostic tests has led to the detection of a number of cases (Autio *et al.*, 1973; Nordén *et al.*, 1973; Tsay *et al.*, 1974; Gehler *et al.*, 1975; Farriaux *et al.*, 1975).

In normal Angus-cattle tissues, α -mannosidase exists in at least three forms separable by chromatography on DEAE-cellulose (Phillips *et al.*, 1974a). Two forms, A and B, have an acidic pH optimum

and a third form, C, has a neutral pH optimum. In plasma another form of α -mannosidase, with a pH optimum of 5.5, intermediate between those of the acidic and neutral activities, occurs (Winchester *et al.*, 1976). The concentration of acidic α -mannosidase is low in the tissues of calves with mannosidosis, less than 10% of the normal activity being present. Whether this residual acidic α -mannosidase in mannosidosis is a decreased amount of the normal enzyme or a modified product of the defective gene is not known. The residual acidic α -mannosidase in human mannosidosis has different properties from the normal enzyme (Hultberg, 1970; Hultberg & Masson, 1975; Beaudet & Nichols, 1976), and it is suggested that it results from a mutation in the structural gene for the enzyme. In the present paper we compare the immunological properties of acidic α -mannosidase in normal bovine tissues and the residual acidic α -mannosidase in the tissues of a calf with mannosidosis by using antiserum that has been raised against acidic α -mannosidase from normal bovine kidney.

Evidence is presented that the residual acidic α -mannosidase in bovine mannosidosis cross-reacts

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with antiserum raised against acidic α -mannosidase from normal bovine tissues. The detection of immunological cross-reacting material in animals with mannosidosis has important implications for the success or otherwise of enzyme-replacement therapy, a particularly useful aspect of an animal model. In the absence of cross-reacting material even bovine acidic α -mannosidase might be rejected by the diseased animals (Boyer *et al.*, 1973). That a calf with mannosidosis can utilize acidic α -mannosidase from normal cells has been shown clinically and morphologically in a chimeric calf that had obtained a natural transplant of normal cells during foetal development (Jolly *et al.*, 1976).

Materials and Methods

Tissue

The tissues from a 3-week-old Angus calf, which has been diagnosed clinically and by histopathology as suffering from mannosidosis, were stored frozen at -20°C until required. The corresponding tissues from a control calf of similar age and post-mortem human kidney were stored under identical conditions. Fresh kidneys from cows were collected from a slaughterhouse for the purification of bovine kidney α -mannosidase. Tissue homogenates (10%, w/v) were prepared in a Potter-Elvehjem homogenizer in the appropriate buffer or in water at 4°C . 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.

α -Mannosidase was assayed by using the fluorogenic substrate 4-methylumbelliferyl α -D-mannopyranoside (Koch-Light Laboratories, Colnbrook, Bucks., U.K.) as described previously (Phillips *et al.*, 1974a). Activity measured at pH4 and pH6.5 was termed acidic and neutral α -mannosidase respectively. The McIlvaine (1921) phosphate/citrate buffer system was used to investigate the pH-dependence of the activity. The effect of EDTA, Co^{2+} and Zn^{2+} on the α -mannosidase activity was investigated by including 1 mM-EDTA, 1 mM- CoSO_4

or 1 mM- ZnSO_4 in the substrate solutions, giving a final concentration of 0.5 mM in the incubation mixture. All reagents were of A.R. grade. One unit of activity is that amount of enzyme that transforms $1\mu\text{mol}$ of substrate/min under these conditions. The kinetic parameters K_m and V were calculated by the graphical method of Lineweaver & Burk (1934) by using a computer program to obtain the line of best fit.

β -D-Glucosidase (EC 3.2.1.21), β -D-galactosidase (EC 3.2.1.23), *N*-acetyl- β -D-glucosaminidase (EC 3.2.1.30) and α -L-fucosidase (EC 3.2.1.51) were assayed during the purification procedure by using the appropriate fluorogenic 4-methylumbelliferyl substrate (Koch-Light) and β -D-mannosidase (EC 3.2.1.25) was assayed by using *p*-nitrophenyl β -D-mannopyranoside (Koch-Light) (Phillips *et al.*, 1975, 1976).

Partial purification of bovine kidney acid α -mannosidase

Acidic α -mannosidase was partially purified from bovine kidney by the same procedure and with the same apparatus used to purify acidic α -mannosidase from human liver (Phillips *et al.*, 1976) except that the final step, affinity chromatography on agarose- ϵ -aminohexanoylfucosylamine to remove contaminating α -L-fucosidase, was omitted. Further details are given in Table 1.

Polyacrylamide-gel electrophoresis

Electrophoresis of samples from the various stages in the purification was carried out in 7.5% (w/v) polyacrylamide gels by using the discontinuous buffer system of Davis (1964). α -Mannosidase activity and proteins were detected on the gel surface as described previously (Phillips *et al.*, 1975).

Preparation of antiserum

Antiserum was raised in a rabbit as described previously (Phillips *et al.*, 1975) by intramuscular injection of a preparation of bovine kidney acidic α -mannosidase containing 4.5 mg of protein and having a specific activity of 0.24 unit/mg of protein.

Table 1. Purification of bovine kidney acidic α -mannosidase
For details see the Materials and Methods section.

Step	Volume (ml)	Protein (mg)	Specific activity (unit/mg of protein)	Purification (fold)	Yield (%)
Homogenate	1500	55000	0.00042	1	100
					(23.1 units)
Supernatant (25000g, 30 min)	1000	30000	0.0006	1.4	78
Precipitate 40–60%-satd. $(\text{NH}_4)_2\text{SO}_4$	320	4100	0.0034	8.1	60
Chromatography on concanavalin A–Sepharose	60	200	0.067	160	58
Chromatography on Sepharose 4B– ϵ -amino-hexanoylmannosylamine	5	6	0.24	571	6
					(1.44 units)

Immunoprecipitation experiments

(1) *Quantitative immunoprecipitation of α -mannosidase.* A constant amount (50 μ l) of the antigen (diluted 20-fold in 0.15M-NaCl in 10mM-sodium phosphate buffer, pH 7.0) or of the supernatant from a 10 or 20% (w/v) homogenate of a normal Angus-calf tissue extract in the phosphate-buffered saline was placed in a series of 15ml conical centrifuge tubes. For precipitation of the residual α -mannosidase in the pathological liver the volume of the supernatant was increased to 200 μ l. Then 0–100 μ l of diluted (10- or 20-fold) antiserum was added to the tubes and the volume made up to 300 μ l with the phosphate-buffered saline. After incubation at 4°C for 16h the tubes were centrifuged at 1500g for 15 min. The supernatant was removed and 200 μ l assayed for α -D-mannosidase activity at pH 4.0 and 6.5 by the normal procedure. The percentage of α -mannosidase activity remaining in the supernatants was plotted against the volume of antiserum in the incubation mixture per unit of enzyme activity (Pollock, 1963). To detect the presence of cross-reacting material in the tissues of the calf with mannosidosis, 50 μ l of the supernatant from a 10% (w/v) homogenate of the pathological organ was added to 50 μ l of a comparable homogenate from the corresponding normal organ in the incubation mixture and titrated against various dilutions of the antiserum as described above.

(2) *Antibody consumption.* Samples (0–75 μ l) of the supernatants from a 10% (w/v) homogenate of the normal and corresponding pathological organs were incubated at 4°C for 16h with 100 μ l of 5-fold diluted antiserum in a final volume of 200 μ l. Samples (100 μ l) of the supernatants obtained by centrifugation of the incubation mixtures at 1500g for 15min were subsequently re-incubated at 4°C for 16h with 50 μ l of the extract of the normal organ. The acidic α -mannosidase activity remaining in the supernatant obtained by centrifuging the second incubation mixture at 1500g for 15 min was measured. The α -mannosidase activity precipitated by the samples of antiserum that had been preincubated was calculated as a percentage of the activity precipitated by an identical sample of the antiserum that had not been preincubated with a tissue extract. The capacities of the normal and pathological tissue extracts to remove antibodies in the preincubation were expressed as the percentage decrease in α -mannosidase precipitated by the treated samples compared with the untreated sample of antiserum.

Immunodiffusion

Double diffusion of the antigen and extracts of normal and pathological kidneys against the antiserum was carried out as described previously (Phillips *et al.*, 1975).

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Protein determination

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

Results

Partial purification of bovine kidney acidic α -mannosidase

Bovine kidney acidic α -D-mannosidase was purified 570-fold by a procedure involving chromatography on concanavalin A-Sepharose and affinity chromatography on Sepharose 4B- ϵ -aminohexanoyl-mannosylamine (Table 1). A very similar procedure has been used for the purification of human liver acidic α -mannosidase (Phillips *et al.*, 1976). Polyacrylamide-gel analysis of the preparation after affinity chromatography showed that it consisted of six protein bands, only one of which possessed α -D-mannosidase activity (Fig. 1). The only detectable glycosidase contaminant in the preparation was α -L-fucosidase

Fig. 1. Polyacrylamide-gel electrophoresis of partially purified bovine kidney α -mannosidase (a) Protein components; (b) α -mannosidase activity. Details of electrophoresis and staining methods are described in the Materials and Methods section; 20 μ l of purified α -mannosidase was analysed.

(0.02 unit/mg of protein) compared with 0.24 unit of α -mannosidase/mg of protein. The purified α -mannosidase had a pH optimum of 4.25 and did not contain any neutral α -mannosidase. The neutral α -D-mannosidase, in contrast with the acid activity, was not adsorbed on the concanavalin A-Sepharose column and was therefore removed from the preparation before the affinity-chromatography step. This was essential, because the neutral activity binds more strongly to the affinity ligand and competes successfully with the acidic activity (Robinson *et al.*, 1975).

The pH optimum of the partially purified α -D-mannosidase was lowered to pH 4.0 in the presence of 0.5 mM- Zn^{2+} and there was a 70% increase in activity at this pH. The activity in a crude kidney extract responds similarly (Phillips *et al.*, 1974a). In contrast, 0.5 mM-EDTA had no effect on the activity of pH optimum, whereas it inhibited the unpurified enzyme markedly below pH 4.5. The K_m of the purified enzyme towards the substrate 4-methylumbelliferyl α -D-mannopyranoside was 0.5×10^{-3} M at pH 4.25, as determined by the graphical method of Lineweaver & Burk (1934). The specific activity of the preparation, based on the theoretical V , was 0.30 unit/mg of protein.

Specificity of the anti-(bovine kidney α -mannosidase) serum

The antiserum raised against the purified bovine kidney α -mannosidase precipitated 85% of the α -D-mannosidase from a solution of the antigen (Fig. 2). The acidic α -mannosidase was also precipitated from a crude extract of a normal bovine kidney (Fig. 3). There was some apparent precipitation of neutral α -mannosidase at higher concentrations of antiserum. However, this is due to the precipitation of the acidic α -mannosidase, which is active at pH 6.5 and accounts for a large proportion of the α -mannosidase activity measured at that pH in a tissue extract. The activity of pH 6.5 not precipitated by the antiserum is due to the true neutral activity. Confirmation that neutral α -mannosidase is not precipitated by anti-(acidic α -mannosidase) serum was obtained by treating the neutral α -mannosidase that was unabsorbed on concanavalin-A Sepharose during the purification with the antiserum. No activity at pH 6.5 was precipitated in the absence of the acidic activity.

The antiserum also precipitated over 95% of the acidic α -D-mannosidase from extracts of normal bovine liver, lymph nodes and spleen and 82% from the brain. Less than 5% of the neutral activity was precipitated from solution from any of the tissue extracts.

Human kidney acidic α -mannosidase cross-reacts partially with the anti-(bovine acidic α -mannosidase) serum (Fig. 3). A small proportion of the activity

Fig. 2. Immunoprecipitation of partially purified bovine kidney acidic α -mannosidase by anti-(bovine acidic α -mannosidase) serum

Details of the immunization and assay procedures are described in the Materials and Methods section. Various volumes of the antiserum and a fixed volume of the enzyme preparation were incubated together in the phosphate-buffered saline, pH 7.0. The supernatants obtained on centrifugation of the incubation mixtures were assayed for α -mannosidase activity at pH 4.0 (●).

at pH 6.5 was precipitated by the antiserum, but again this can be attributed to removal of the acidic activity.

Double diffusion (Ouchterlony) of the antigen and an extract of a normal kidney against the antiserum resulted in the formation of a continuous immunoprecipitin line with α -D-mannosidase activity when the two samples were placed in adjacent wells (Fig. 4). No spur formation was visible. When an extract of a kidney from the calf with mannosidosis was diffused against the antiserum, several immunoprecipitin lines were revealed by staining for protein, but none of the lines possessed α -D-mannosidase activity or corresponded to the band of activity obtained with extracts of a normal kidney.

Investigation into cross-reacting material in tissues from the calf with mannosidosis

There are low concentrations of residual acidic α -D-mannosidase in all the tissues of calves with

Fig. 3. Immunoprecipitation of the α -mannosidase activity in supernatants of bovine and human kidneys. Details are as for Fig. 2 except that the supernatants were assayed at pH 4.0 (●, bovine kidney; ○, human kidney) and at pH 6.5 (■, bovine kidney; □, human kidney).

Fig. 4. Immunodiffusion between anti-(bovine acidic α -mannosidase) serum and the antigen and extracts from the kidneys of a normal calf and a calf with mannosidosis. The precipitin arcs were stained for α -mannosidase with 1-naphthyl α -D-mannopyranoside. As, anti-serum; (1) and (4), antigen; (2), normal calf kidney; (3), kidney from calf with mannosidosis.

mannosidosis (Hocking *et al.*, 1972; Phillips *et al.*, 1974a; Jolly *et al.*, 1976). The acidic activities in the control calf and the calf with mannosidosis used in this investigation are given in Table 2. If the residual acidic activity in the calf with mannosidosis is the product of the defective gene, i.e. an altered enzyme, it might have different antigenic determinants from the normal enzyme. Therefore experiments were designed to investigate whether the residual acidic activity in the calf with mannosidosis cross-reacted with the antiserum raised against the normal bovine acidic α -D-mannosidase. First, the immunoprecipitation curve for the activity in a normal tissue extract was re-determined in the presence of an equal volume of a similar extract from the corresponding pathological organ. If the antigenicity of the residual acidic activity was different, or if cross-reacting but enzymically inactive material was present in the sample from the calf with mannosidosis, the immunoprecipitation curve would be different from that obtained for the normal extract

Table 2. Acidic α -mannosidase activity in the tissues from the calf with mannosidosis and from a control calf. α -Mannosidase was assayed at pH 4.0 in the supernatant obtained after centrifugation of a 10% (w/v) homogenate at 37000g for 15 min.

	Activity (munits/g wet wt. of tissue)	
	Control	Mannosidosis
Brain	20.3	1.4
Kidney	59.6	2.5
Liver	55.6	9.7
Lymph nodes	43.7	6.65
Spleen	32.2	5.2

Fig. 5. Immunoprecipitation of the acidic α -mannosidase in normal calf tissues and in mixtures of the tissues from a normal calf and a calf with mannosidosis. Details are as for Fig. 2. ●, Acidic α -mannosidase in extracts of normal calf tissues; ○, acidic α -mannosidase in an equal mixture of the extract of a normal calf tissue and of the extract of the corresponding organ in the calf with mannosidosis. (a) Kidney; (b) lymph node; (c) spleen; (d) liver.

Fig. 6. Consumption of antibodies against bovine acidic α -mannosidase by extracts of the tissues of a normal calf and of a calf with mannosidosis

Various volumes of the supernatants from tissues of the calf with mannosidosis and of the control calf were preincubated with a fixed volume of the antiserum. After centrifugation, the supernatant was incubated with a fixed volume of the supernatant of the normal tissue. The activity remaining in the supernatant obtained on centrifugation of the second incubation mixture was removed. The proportion of antibodies removed in the preincubation was then calculated as described in the Materials and Methods section. ●, Percentage of antibodies consumed by normal tissue extract in preincubation; ○, percentage of antibodies consumed by pathological tissue extract in preincubation. (a) Liver, (b) spleen and (c) lymph nodes.

alone. The mixing of the extracts from the normal and pathological organs did not result in any increase in enzymic activity. Fig. 5(a) shows that the immuno-precipitation curves for the kidney extracts are superimposable. It is concluded that the residual activity in the pathological kidney is precipitated by the antiserum and that the same volume of antiserum is required to precipitate a unit of activity from the pathological kidney as is required to precipitate a unit of activity from the normal kidney. Further, it is unlikely that there is a high concentration of enzymically inactive but cross-reacting material in the pathological kidney.

This technique is applicable to other organs, since the antiserum precipitated acidic α -D-mannosidase from all the normal tissues examined. The immuno-precipitation curves for a mixture of normal and pathological lymph nodes and for the normal organ alone were also superimposable (Fig. 5b). In contrast the curves for the mixtures of normal and pathological spleens or livers (Figs. 5c and 5d) were slightly different from those of the normal organs, suggesting either that the antigenicity of the acidic α -mannosidase in the pathological organs is different from that of the activity in the normal organs or that enzymically inactive but cross-reacting material is present.

A quantitative but indirect method, antibody

consumption, was also used to detect cross-reacting material in the tissues of the calf with mannosidosis. The capacities of extracts of normal and pathological tissues to remove antibodies from the antiserum were compared by preincubating the antiserum separately with the two tissue extracts before using it to precipitate α -D-mannosidase from another sample of the normal tissue. The presence of antigen or cross-reacting material in the preincubation should decrease the concentration of antibodies in the antiserum. Cross-reacting material was detected in all the pathological organs except the kidney by this technique and the highest concentration was again found in the liver (Fig. 6). From such antibody-consumption curves it is possible to compare the concentration of cross-reacting material in the pathological organ with the concentrations of antigen in the corresponding normal organ. The concentration of cross-reacting material in the tissues of the calf with mannosidosis appeared to be proportional to the concentration of residual acidic α -mannosidase. This suggests that the residual acidic activity accounts for the cross-reacting material in these pathological organs and that enzymically inactive but cross-reacting material is absent.

To test whether the residual α -mannosidase in mannosidosis has the same antigenicity as the normal

Fig. 7. Immunoprecipitation of the acidic α -mannosidase in normal calf liver compared with immunoprecipitation of the residual acidic α -mannosidase in the liver of the calf with mannosidosis

Details are as for Fig. 2. Acidic α -mannosidase in normal calf liver (●) and in liver of calf with mannosidosis (○).

enzyme, direct immunoprecipitation of the residual activity in the liver of the calf with mannosidosis was carried out. The liver was chosen because this organ has the highest concentration of residual activity, and antibody-consumption experiments showed that the concentration of cross-reacting material appeared to be relatively higher in this organ than in the other pathological organs. The immunoprecipitation curves for the activity in the normal and pathological livers are essentially superimposable (Fig. 7), suggesting that the mutant enzyme has the same antigenicity as the normal enzyme and that enzymically inactive but cross-reacting material is not likely to be present.

Discussion

The antiserum raised against the purified bovine kidney acidic α -mannosidase precipitated acidic α -mannosidase from all the bovine tissues, suggesting that the enzyme does not exist in immunologically distinct organ-specific forms. It precipitated over 95% of the acidic activity from liver, lymph nodes and spleen, implying that both A and B forms of the acidic α -mannosidase separable by chromatography on DEAE-cellulose (Phillips *et al.*, 1974a) must have reacted. Neutral α -mannosidase did not cross-react with the antiserum. Thus anti-(bovine acidic α -mannosidase) serum has the same specificity toward bovine α -mannosidases as anti-(human acidic α -

mannosidase) serum has towards human α -mannosidases (Phillips *et al.*, 1975). However, the anti-(human acidic α -mannosidase) serum did not cross-react with the bovine enzyme, whereas we have shown that the anti-(bovine acidic α -mannosidase) serum does cross-react partially with the human enzyme. Immunological asymmetry also occurs in mixed leucocyte reactions (Widmer & Bach, 1972). de Barsey *et al.* (1972) found partial cross-reaction between lysosomal α -glucosidases from different species. Bovine and human acidic α -mannosidase also have very similar physicochemical properties (Phillips *et al.*, 1974a,b). Thus if experimental enzyme-replacement therapy were ever contemplated for human mannosidosis patients, bovine tissues might be a more convenient source of the enzyme. The partial cross-reactivity increases the relevance of bovine mannosidosis as an animal model for the human disease.

That there is a product of the defective gene in mannosidosis is shown by the low concentrations of residual acidic α -mannosidase in the tissues of the calf with mannosidosis. The concentration of residual activity relative to the concentration of activity in the corresponding normal tissues varies from organ to organ and from diseased animal to animal (Hocking *et al.*, 1972; Jolly *et al.*, 1976). A partially functional product of the defective gene or a low concentration of the normal gene product could account for this residual activity. The immunological properties of the activity in the pathological tissues were investigated by using the antiserum raised against the product of the normal gene. When an extract of a pathological tissue was added to an extract of the corresponding normal organ, no change in the immunoprecipitation curve for α -mannosidase was observed for the kidney and lymph nodes. This showed that the residual acidic activity in these pathological organs was precipitated by the antiserum and required the same volume of antiserum to precipitate a unit of enzyme activity as the normal enzyme. Therefore either a decreased concentration of the normal gene product with normal specific enzymic activity and antigenicity or an altered enzyme with a decreased specific enzymic activity and a correspondingly decreased antigenicity could account for the residual acidic α -mannosidase in these pathological organs. The correlation between the decreased activity and antigenicity could be fortuitous or as a result of specific alterations in the structure. Both interpretations preclude the presence of a high concentration of enzymically inactive but fully cross-reacting material in these organs. However, the immunoprecipitation curves for the α -mannosidase in the mixture of the normal and pathological livers and spleens were slightly different from the curves for the activity in the normal liver and spleen alone. Therefore it is possible that the residual acidic

α -mannosidase has a different antigenicity from the normal enzyme or that there is a low concentration of enzymically inactive but cross-reacting material in the pathological organs.

As the pathological extracts only contributed a small proportion of the total activity in the mixtures used in the immunoprecipitation experiments, the concentration of activity with an altered antigenicity might have been insufficient in the cases of the kidney and lymph nodes to produce a detectable difference in the immunoprecipitation curves. Therefore the technique of antibody consumption was also used to investigate cross-reacting material in the pathological tissues. Cross-reacting material was detected in all the pathological organs except the kidney by this technique, with the highest concentration occurring in the liver of the calf with mannosidosis. The concentration of cross-reacting material appeared to be proportional to the concentration of residual acidic α -mannosidase. The inability to detect cross-reacting material in the pathological kidney was probably due to the low concentration of residual acidic α -mannosidase in that organ. The results of the antibody-consumption experiments and the immunoprecipitation of the activity in mixtures of normal and pathological tissue extracts suggest that the residual acidic α -mannosidase accounts for the cross-reacting material in the pathological organs and that enzymically inactive but cross-reacting material is absent.

Cross-reacting but enzymically inactive material has been demonstrated in several human lysosomal storage diseases, namely metachromatic leucodystrophy (Neuwelt *et al.*, 1971), GM₁-gangliosidosis (Meisler & Rattazzi, 1974), and Sanfilippo disease type B (von Figura & Kresse, 1976). There are conflicting reports for the presence (Romeo *et al.*, 1975) and absence (Rietra *et al.*, 1974) of cross-reacting material in Fabry's disease, but no cross-reacting material has been detected in Pompe's disease (de Barsey *et al.*, 1972). In the GM₂-gangliosidosis no material that carries hexosaminidase A-specific antigenic determinants has been found in Tay-Sachs disease, but inactive cross-reacting material has been detected in Sandhoff's disease (Srivastava & Beutler, 1973; Geiger *et al.*, 1975). In addition, Carroll & Robinson (1974) have detected cross-reacting material of a low molecular weight in the livers of patients of both diseases. The mutant enzyme responsible for the residual arylsulphatase B activity in the Maroteaux-Lamy syndrome has identical antigenic determinants with the normal enzyme but only 15% of the specific activity (Shapira *et al.*, 1975).

Direct immunoprecipitation of the residual acidic α -mannosidase in the liver of the calf with mannosidosis showed that the mutant enzyme has the same antigenicity as the enzyme in normal bovine liver. Therefore it is concluded that in bovine mannosidosis the residual acidic α -mannosidase accounts for all the

cross-reacting material detected. Whether the residual enzymic activity is a decreased concentration of the normal gene product or an altered enzyme with a decreased specific enzymic activity and correspondingly decreased antigenicity cannot be resolved by the serological techniques described here with crude tissue extracts. Purification of the products of the normal and defective genes will resolve the question.

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