Uptake of Iodinated Human Kidney α -D-Mannosidase by Rat Liver ASSOCIATION WITH MEMBRANE ELEMENTS AND STABILITY *IN VIVO* AND *IN VITRO*

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1. Human kidney α -D-mannosidase (form A) was labelled with ^{125}I to a specific radioactivity of approx. $2250\ \mu\text{Ci}/\text{mg}$ of protein, essentially without loss of enzymic activity. The enzymic activity and radioactivity of the iodinated material also co-migrated in gel filtration and gel electrophoresis. 2. The binding of ^{125}I -labelled mannosidase *in vitro* to particulate material in liver and kidney homogenates was of the order of $2\ \text{pg}/\text{mg}$ of particulate protein within 16 h at 37°C , and essentially zero in intervals of up to 60 min. The degradation *in vitro* of labelled exogenous mannosidase was of the order of $10\text{--}20\ \text{pg}/16\ \text{h}$ per mg of protein in postnuclear supernatant, and it was saturated entirely within 1 h at 37°C . 3. The binding of labelled mannosidase *in vivo* to particulate elements of liver homogenates 60 min after intravenous injection was at least 10 times higher in terms of specific radioactivity than the highest value attainable *in vitro*. Virtually all exogenous enzyme bound to liver particulate material could be recovered in macromolecular form after disruption of membranes by detergents. 4. The radioactive enzyme bound to liver particulate material could be detached almost completely by shearing, repeated freezing and thawing, and exposure to strong detergents under conditions that do not eliminate rough-endoplasmic-membrane structure. It could not be released, however, by high salt concentration ($0.5\ \text{M-KCl}$) or by exposure to weak detergents such as Tween 80. The particle-bound enzyme should thus be associated with plasma membranes and lysosome-like elements. 5. Of the rat tissues studied, only liver could approach, within 60 min after the injection, the concentration of exogenous mannosidase found in the blood serum. The activity per g tissue weight fell progressively from liver (60% of serum value) to kidney (16% of serum value), lung (8% of serum value), spleen (6% of serum value) and brain (0.9% of serum value). Most of the radioactive enzyme found in tissues other than liver appeared to be present in a free form, whereas in liver more than 50% of the labelled enzyme was associated with membrane elements.

Deficiency of lysosomal glycosidases has been implicated or demonstrated in a number of inherited mammalian disorders, including forms of mannosidosis (Öckerman, 1967; Hocking *et al.*, 1972; Carroll *et al.*, 1972; Phillips *et al.*, 1974a; Taylor *et al.*, 1975; Tsay & Dawson, 1975). These lysosomal enzymes are known to be readily taken up by cultured mammalian cells, and to be capable of exerting enzyme activity inside the recipient cells (Kihara *et al.*, 1973; Neufeld, 1973; Brot *et al.*, 1974). Such supplementation should also be feasible in living mammals, in view of known high blood-clearance rates of a variety of glycoproteins (Morell *et al.*, 1971), including glycosidases (Brady *et al.*, 1974). However, no detailed account of the disposition and fate has been published so far for any of the more common

lysosomal glycosidases in mammals. Such a study should be useful in general biochemical and physiological terms, and also with regard to prospects of enzyme-supplementation therapy in glycosidase disorders.

In studying mechanisms of exogenous glycosidase uptake and utilization in mammalian cells and tissues, it is particularly important to establish possible tissue and cell-type preferences in their disposition, and also the extent and selectivity of their association with subcellular compartments or structures. To study this, we chose the A form (Phillips *et al.*, 1974a,b) of mammalian α -mannosidase, one of the glycosidases well-identified with deficiency diseases (Carroll *et al.*, 1972; Nordén *et al.*, 1973; Phillips *et al.*, 1974b; Taylor *et al.*, 1975). This enzyme is also readily prepared in a highly purified form by either conventional techniques (Marinković & Marinković, 1976)

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or an affinity-chromatography approach (Robinson *et al.*, 1975). It is fairly stable to denaturation (Phillips *et al.*, 1974a), and is thus able to persist in an enzymically active form during the iodination process that is necessary for its radioactive labelling.

Experimental

Materials

Radioactive material. Na¹²⁵I (carrier-free; 600 mCi/ml at the beginning of each iodination experiment) was purchased from NEN (Boston, MA, U.S.A.).

Chemicals. Bio-Gel P-60 was made by Bio-Rad Laboratories (Richmond, CA, U.S.A.). Sephadex G-200, Sepharose 4B and Blue Dextran 2000 were obtained from Pharmacia (Uppsala, Sweden). Chemicals for polyacrylamide-gel electrophoresis were purchased in a kit form from Canalco (Bethesda, MD, U.S.A.). *p*-Nitrophenyl α -D-mannoside was bought from Sigma (St. Louis, MO, U.S.A.). Chloramine-T (sodium *N*-monochlorotoluene-*p*-sulphonate) was a product of Eastman Kodak (Rochester, NY, U.S.A.). Triton X-100 [alkylphenoxy-polyethoxy(10)ethanol] and Tween 80 [polyoxyethylene(20) sorbitan mono-octenoate] were purified technical-grade chemicals, supplied by Sigma. All other chemicals used were A. R. grade.

Animals and treatments. Sprague-Dawley female rats, weighing 200–210 g, were supplied by Holtzman (Madison, WI, U.S.A.). The animals were injected via the femoral vein with 0.1 ml (approx. 2 μ g or 1×10^7 c.p.m.) of ¹²⁵I-labelled α -mannosidase from human kidney (form A). Approx. 2 ml of blood was withdrawn from the heart 60 min later. This blood was allowed to clot at room temperature (20°C), and was then centrifuged to obtain serum for radioactivity assays. The animals were killed by decapitation after cardiac puncture, immediately after collection of blood. Liver, kidneys, spleen, lung and brain were promptly removed, washed with cold 0.9% NaCl, blotted and weighed.

Methods

Purification of α -D-mannosidase from human kidney was done as described by Marinković & Marinković (1976). The purified form A migrated as a single band on polyacrylamide-gel electrophoresis (see also the Results section, Fig. 1). The enzyme was purified approx. 2100-fold over the crude tissue homogenate, to a specific activity of about 10 units (Marinković & Marinković, 1976) per mg of protein.

Iodination of mannosidase. This was done by a modification of the method of Hunter & Greenwood (1962). Solutions of the enzyme (0.1 mg in 0.25 ml), chloramine-T (3 mg/ml) and sodium metabisulphite (3 mg/ml) were prepared fresh, in 0.05 M-sodium phosphate buffer (pH 7.5). Na¹²⁵I (4.6 μ l; 1.5 mCi)

was added to the enzyme solution, diluted with 0.25 μ l of the same buffer, mixed with 10 μ l (30 μ g) of chloramine-T, and shaken gently for 75 s. The reaction was then stopped by adding 50 μ l (0.15 mg) of the above bisulphite solution. The solution was promptly loaded on a column (13 cm $\times$ 0.9 cm diam.) of Bio-Gel P-60 pre-equilibrated with 0.15 M-NaCl containing 0.001 M-sodium phosphate buffer (pH 7.5). Fractions (0.4 ml) were collected, and their radioactivity was determined. Fractions corresponding to the peak of radioactivity emerging from the column in the void volume were combined, and after re-determination of radioactivity and enzyme activity were used for experimental treatments.

Radioactivity assays. These were done with a Nuclear-Chicago model 1185 gamma scintillation spectrometer, in 10 mm $\times$ 75 mm plastic tubes. Gel columns were sliced in to 2 mm-thick sections before radioactivity determination. Tissue samples were weighed in the tubes used for the counting.

Electrophoresis. Polyacrylamide-gel (7.5%, w/v) electrophoresis was carried out in 0.75 M-Tris/HCl buffer (pH 8.9) at 3 mA per gel for 3 h at 4°C, by the procedure of Davis (1964). The mannosidase activity was assessed in homogenates made in the substrate solution (Marinković & Marinković, 1976) from 2 mm-thick gel sections.

Tissue homogenization and fractionation. The weighed organs were minced in 4 vol. of ice-cold 0.25 M-sucrose/0.01 M-MgCl₂/0.01 M-Tris/HCl buffer (pH 7.6). The minced tissue was dispersed with a loosely fitting (0.30 mm minimum clearance) pestle of the homogenizer designed by Philpot & Stanier (1956), by applying 30–40 up-and-down strokes. The suspension was filtered to remove the fibrous tissue debris, and the filtrate was homogenized with 50 strokes of the tight-fitting (0.12 mm minimum clearance) pestle of the above homogenizer. The homogenates were then centrifuged twice for 9 min at 2500 g_{max} in a Sorvall SS-1 centrifuge at 4°C. The second supernatant was further characterized by gradient centrifugation and gel filtration.

Particulate elements were isolated from void-volume effluents of gel-electrophoresis runs, or from other sources, by centrifugation for 60 min at 40 000 rev./min (240 000 g_{max}) in a no. 60 Ti rotor of the Spinco L5-65 ultracentrifuge. After sedimentation, the clear supernatants could be freely decanted from the firm sediments, which were carefully drained before resuspension.

Gel-filtration analysis. Gel-filtration analysis of the tissue homogenates with respect to binding of mannosidase to particulate elements or to its degradation by tissue proteinases was done with columns (35 cm $\times$ 2.2 cm diam.) of Sepharose 4B equilibrated with the tissue-homogenization medium (see above). About 2–4 ml of the above supernatants of tissue homogenates containing labelled mannosidase could

be satisfactorily resolved into three principal zones (A, B and C in Fig. 2) corresponding to, respectively, the particle-attached enzyme (in the void volume), free native enzyme (in the K_D region of 0.35–0.70) and the degraded enzyme plus radioactive low-molecular-weight material (in the total column volume zone). The distribution coefficients were calculated as described by Flodin (1961).

Sucrose-density-gradient centrifugation. This was carried out essentially as outlined by Martin & Ames (1961), with linear gradients from 20 to 60% (w/v) sucrose made in a buffer containing 0.025 M-KCl, 0.01 M-MgCl₂ and 0.02 M-Tris/HCl (pH 7.6). Samples of up to 0.6 ml were layered on top of the gradients (precooled to 4°C) and centrifuged for specified intervals in the SW65 Ti rotor of a Spinco L5-65 ultracentrifuge. Fractions of volume about 0.15 ml were collected through the tube bottoms, and stored at 4°C until assayed.

Results

Stability of mannosidase to iodination and towards tissue-proteolytic activities in vitro

After iodination under the conditions specified in the Experimental section, human kidney α -D-mannosidase exhibited approximately the same speci-

fic activity as the unmodified enzyme, about 9.5 units (Marinković & Marinković, 1976) per mg of enzyme protein. The iodinated enzyme protein also comigrated with the mannosidase activity on polyacrylamide-gel electrophoresis (Fig. 1a) and on Bio-Gel P-60 filtration assays (Fig. 1b).

The iodinated enzyme appeared to be rather resistant to degradation by proteinases of rat tissue homogenates *in vitro*. Thus when incubated for 16 h at 37°C, not more than 0.2 ng of enzyme/mg of protein could be converted into low-molecular-weight material in the 2500g supernatants from either liver or kidney homogenates (Table 1). Most of the degradation with either tissue homogenate seemed to occur in a short interval after addition of the enzyme, as if the proteolytic activity was saturated and inhibited by the amount of enzyme used.

The association *in vitro* of exogenous mannosidase with membrane elements in the 2500g supernatants from liver and kidney was very slight, about 20 pg/mg of particulate protein from either source, over a 16 h interval at 37°C (Table 1).

Distribution of iodinated mannosidase in rat tissues after intravenous injection

A survey of the distribution of ¹²⁵I-labelled human kidney α -mannosidase (form A) in various tissues

Fig. 1. Characterization of iodinated human kidney α -D-mannosidase (form A) by gel filtration and electrophoresis

(a) Bio-Gel P-60 filtration of the iodinated enzyme. An 8 ml column of Bio-Gel P-60 was used as specified in the Experimental section. Fractions (0.4 ml) were collected. $\circ$, α -Mannosidase activity (units/10 min per ml); $\bullet$, ¹²⁵I radioactivity. The solid bar indicates the fractions pooled. (b) Polyacrylamide-gel electrophoresis of the iodinated enzyme. $\bullet$, Radioactivity; $\blacktriangle$, α -mannosidase activity, units (Marinković & Marinković, 1976)/14 h incubation per 2 mm gel. The direction of the cathode is toward the left. The electrophoresis was carried out as described in the Experimental section.

Table 1. *Degradation of iodinated heterologous mannosidase by rat tissue homogenates*

The supernatants (2500 g_{max} ; 2 ml) of liver and kidney homogenates (containing respectively about 60 and 45 mg of total protein/ml) were incubated at 37°C with 0.02 ml (2×10^5 c.p.m., or 0.4 ng) of the iodinated human kidney α -D-mannosidase (form A) for the indicated periods. The incubation was ended by quick chilling to approx. 0°C, and the chilled mixtures were immediately loaded on Sepharose 4B columns at 4°C; for details of gel filtration see the legend to Fig. 2. The void-volume material included fractions corresponding to K_D range 0–0.2; the free-mannosidase zone corresponded to K_D values between 0.28 and 0.70, and the total-column-volume zone included the K_D 0.8–1.15 material. The enzyme preparation used carried less than 1% of its radioactivity in this zone.

Chromatographic fraction	Time of incubation (h) ...	Percentage of total load recovered in:					
		Liver			Kidney		
		0	1	16	0	1	16
Void-volume zone		0	0	2.9	0	0	2.8
Free-enzyme zone		71.4	58.0	56.7	68.1	58.6	56.0
Total-volume zone		28.6	42.0	40.4	31.9	41.4	41.2
$10^{-3} \times$ Degraded enzyme (μ g/mg of protein)		0.0977	0.1435	0.1380	0.1450	0.1885	0.1870

Table 2. *Distribution of iodinated heterologous mannosidase in some rat organs 60 min after intravenous injection*

The data are algebraic means from five experiments on single animals, with a body weight of 210 ± 1.5 g. All data are expressed with the respective standard error, $\sqrt{(x - \bar{x}^2)/n(n-1)}$. The animals were each intravenously injected with 1.009×10^7 c.p.m. of the iodinated human α -D-mannosidase (form A), corresponding to 2.07 μ g of radioactive enzyme. Serum radioactivity was calculated by assuming a blood content of 5% of the body weight, and a haematocrit value of 50%.

Organ	$10^{-3} \times$ Total radioactivity (c.p.m./organ)	Mean tissue wt. per animal (g)	$10^{-3} \times$ Radioactivity (c.p.m./g of tissue)	Percentage of total injected radioactivity
Liver	2924 ± 83.1	7.01 ± 0.12	417.7 ± 14.4	29.0 ± 0.8
Kidney	167.9 ± 6.5	1.51 ± 0.05	111.1 ± 3.4	1.7 ± 0.1
Lung	67.5 ± 6.3	1.17 ± 0.06	57.5 ± 3.7	0.7 ± 0.1
Serum	3638 ± 99.9	5.26 ± 0.1	691.1 ± 20.5	36.4 ± 1.0
Spleen	79.3 ± 9.8	0.50 ± 0.01	39.7 ± 5.2	0.4 ± 0.06
Brain	12.7 ± 0.8	2.04 ± 0.01	6.23 ± 0.4	about 0.1

60 min after intravenous injection into the rat is presented in Table 2.

At this interval after injection, next to blood, most of the administered radioactivity was associated with liver. The activity per tissue weight indicated that liver alone effected a significant accumulation of the radioactive material. All other organs exhibited at this interval specific radioactivities far below those found for blood serum or for liver. In view of this result, further work was concentrated on liver as a primary 'target' for the exogenous enzyme.

After 1 h of the incorporation *in vivo*, nearly all the radioactivity in the 2500g supernatants of homogenates of several rat tissues could be recovered (after disruption of particulate elements by detergents) in a form co-filtering with its enzyme activity on Sepharose 4B (as exemplified in Fig. 1b). The degradation of the exogenous enzyme *in vivo* thus did not appear to be significant in the interval studied.

Nearly 60% of mannosidase radioactivity associated with the liver after 1 h of enzyme incorporation

in vivo was in the particulate fraction of the 2500g supernatant, eluted from Sepharose 4B in the void volume (Fig. 2a); in the kidney supernatant, not more than one-tenth of the radioactive material could be isolated in this fraction (Fig. 2b) whereas lung tissue appeared not to contain any membrane-bound radioactivity in its 2500g supernatant (Fig. 2c). Liver membrane elements concentrated about 20 times more enzyme, on the basis of radioactivity/mg of protein, than did kidney membrane fragments, whereas the exogenous enzyme in lung was probably mostly associated with residual blood.

The particle-associated radioactivity found in liver could be shown, after membrane solubilization, to consist entirely of material with a distribution coefficient on Sepharose chromatography similar to that of the free iodinated enzyme (compare Figs. 2 and 3). No low-molecular-weight degradation products could be detected, although some spreading and irregularity of elution profiles could be detected (Fig. 3), possibly indicating partial alteration in

Fig. 2. Agarose-gel filtration of postnuclear supernatants from rat tissue homogenates prepared after 60 min of incorporation *in vivo* of human kidney α -D-mannosidase (form A)

(a) Liver, (b) kidney, (c) lung 2500 $g_{\max}$ supernatants. Sepharose 4B columns (133 ml) were used; fractions of volume approx. 3 ml were collected. V_0 and V_t denote respectively the void and total column volumes. Δ , α -Mannosidase activity; $\blacksquare$, ^{125}I radioactivity; $\bullet$, E_{280} .

Fig. 3. Solubilization by detergents of mannosidase bound to liver tissue particulates

The membrane elements were recovered by ultracentrifugation for 60 min at $150000g_{av}$ from the void-volume ($K_D = 0-0.2$) material of Sepharose 4B runs (as exemplified in Fig. 2), resuspended in the homogenization medium and brought to 0.2% sodium deoxycholate and to 0.1% Triton X-100 before gel filtration through another Sepharose 4B column (133 ml total volume) as described in the Experimental section. V_0 and V_t denote the void and total column volumes respectively. ■, Radioactivity; Δ, α-mannosidase activity (as in Fig. 2); ●, E_{280} .

molecular size of the radioactive protein. However, some tailing of endogenous mannosidase activity was also noted under these conditions, and thus the spreading of elution zones could have resulted from factors other than degradation of mannosidase, such as protein-protein or protein-detergent interaction after membrane disruption. It is noteworthy that the distribution coefficient for human kidney α-mannosidase (form A) on Sepharose 4B chromatography was consistently higher by about 0.1 K_D unit than that for rat tissue α-mannosidase activity in homogenates from several organs (Figs. 2 and 3); thus in liver, the K_D value for the endogenous free enzyme was 0.49 ± 0.032 (s.d.), as compared with 0.59 ± 0.036 (s.d.) for the exogenous free enzyme (six determinations of each).

Nature of the association of exogenous mannosidase with liver particulate elements

When particles, isolated by ultracentrifugation from the void volume during Sepharose-gel chromatography, were gently resuspended and resedimented through sucrose density gradients, 60% or more of

the radioactive enzyme banded with the heavier membrane elements, above a density of 1.12 g/cm^3 (Fig. 4a). However, a significant portion of the enzyme appeared to be either present as aggregates with lipoproteins having density below 1.12 g/cm^3 , or entirely released by the low amount of mechanical shear applied during resuspension. Prolonged shearing with a motor-driven Potter-Elvehjem pestle, or repeated freezing and thawing of the resuspension (Bendall & de Duve, 1960), resulted in release of nearly all the radioactively labelled enzyme to this low-density fraction. Nearly all of the released enzyme appeared in the position of the free enzyme (K_D 0.55-0.60) in the Sepharose 4B assay.

When the above resuspension was treated (Fig. 4b) with a strong non-ionic detergent under conditions permitting survival of the rough-endoplasmic-membrane fragments (Kreibich *et al.*, 1973), virtually all the radioactive enzyme was released into a low-density fraction that could not enter 20% sucrose during the centrifugation period used here, which should have permitted a density equilibration of membrane elements (Beaufay *et al.*, 1959, 1964; see the legend to Fig. 4). This treatment also resulted in

Fig. 4. Release of α -mannosidase bound to liver particulates

The membrane elements were recovered by ultracentrifugation (see the Experimental section) from the void-volume zone ($K_D = 0-0.2$) of Sepharose 4B runs (as exemplified in Fig. 2), and resuspended in the homogenization medium, to a concentration of 0.6–0.7 mg of protein (6–7 E_{450} units) per ml. The suspension (0.5 ml) was then (a) applied directly to sucrose gradients, or brought to (b) 0.3% (v/v) final concentration of Triton X-100 or (c) 0.6% (v/v) final concentration of Tween 80 and 0.3% of Triton X-100 in a volume of 0.6 ml, before being loaded on to the gradients. The gradients [linear, 20–60% (w/v) sucrose in 0.01 M-MgCl₂/0.02 M-Tris/HCl, pH 7.6; 4 ml total volume] were sedimented for 65 min at 50000 rev./min in a SW65 Ti rotor of Spinco L5-65 ultracentrifuge, at 4°C. Fractions of volume approx. 0.15 ml were collected through the tube bottoms, and after dilution with 0.5 ml of homogenization medium were assayed for E_{450} (●), 125 I radioactivity per fraction (■) and α -mannosidase activity (Δ) (units/ml) per fraction. For further details, see the Experimental section.

the release of much, but not all, of the endogenous α -mannosidase activity associated with liver membrane elements, a finding consistent with the observations of Dewald & Touster (1973).

Attenuation of the effect of Triton X-100 by mixing it with a mild non-ionic detergent, Tween 80, although nearly completely suppressing the disruption of microsomal membrane elements (as judged from the preservation of bands in the E_{450} profile), could not prevent the release of most of the membrane-associated radioactive enzyme (Fig. 4c). Also, the release of endogenous mannosidase activity appeared to be only slightly lower than after treatment with Triton X-100 alone.

Exposure of the same microsomal material to 0.6% Tween 80 alone, or to 0.5 M-KCl, resulted in no significant changes in bandable radioactive mannosidase or E_{450} compared with untreated microsomal suspensions.

Discussion

Uptake of exogenous mannosidase could be positively demonstrated for liver and kidney, where substantial proportions of the organ-associated radioactive enzyme were attached to membrane elements of tissue homogenates (Fig. 2). Essentially

none of this enzyme-membrane association could be described as artifact of preparation, in view of incubation tests *in vitro* (Table 1), and of the ease of detachment of the labelled enzyme from liver membrane fragments (Fig. 4). However, it is quite possible that much of the apparently free exogenous enzyme found in rat tissues was detached from membranes during preparation of the homogenates.

The density distribution of liver membrane elements bearing exogenous mannosidase appeared to be somewhat heterogeneous (Figs. 4a and 4c), as for lysosomal hydrolases (Beaufay *et al.*, 1959, 1964; Badenoch-Jones & Baum, 1974). Preferential detachment of the exogenous enzyme by detergent mixtures, which essentially could not disrupt endoplasmic membranes (Fig. 4c), also indicated its association with fragile membrane elements. However, the enzyme should be definitely considered to be strongly bound to membranes, if not membrane-enclosed, on the basis of failure of detergents such as Tween 80, and of high salt concentration, to effect any release above the control value. The release of radioactive mannosidase by treatments such as shearing (including ultracentrifugation plus resuspension, as in Fig. 4a) and freezing and thawing corresponds to descriptions of the behaviour of lysosome-bound enzymes (e.g. Bendall & de Duve, 1960). On elimination of

smooth-membrane structure by a strong non-ionic detergent (Fig. 4b), no radioactive enzyme was detected in the rough-microsomal matrix, which was associated with much of the endogenous α -mannosidase activity, in keeping with the report of Dewald & Touster (1973). Thus the exogenous enzyme was confined to non-microsomal elements. Most of the membrane-attached exogenous mannosidase in liver could then be considered, in view of the present data and the experimental evidence and theoretical considerations of Deter (1975a,b), as being part of the vacuolar-lysosomal system involved in protein segregation and compartmentation. Further work, especially with regard to dynamics of the exogenous enzyme passage through the lysosomal apparatus (Deter *et al.*, 1967; Kaulen *et al.*, 1970; Deter, 1971; Davidson *et al.*, 1971), is needed to characterize this association more clearly. However, it appears that there exists a considerable potential for uptake of the exogenous enzyme in at least one important rat organ, the liver, and that the absorbed enzyme largely shares the intracellular localization of endogenous mannosidase.

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References

- Badenoch-Jones, P. & Baum, H. (1974) *FEBS Lett.* **43**, 227–230
- Beaufay, H., Bendall, D. S., Baudhuin, P., Wattiaux, R. & de Duve, C. (1959) *Biochem. J.* **73**, 628–736
- Beaufay, H., Jacques, P., Sellinger, O. E., Berthet, J. & de Duve, C. (1964) *Biochem. J.* **92**, 184–205
- Bendall, D. S. & de Duve, C. (1960) *Biochem. J.* **74**, 444–450
- Brady, R. O., Pentchev, P. G., Gal, A. E., Hibbert, S. R. & Dekaban, A. S. (1974) *N. Engl. J. Med.* **291**, 989–993
- Brot, F. E., Glaser, J. H., Roozen, K. J., Sly, W. S. & Stahl, P. D. (1974) *Biochem. Biophys. Res. Commun.* **57**, 1–8
- Carroll, M., Dance, N., Mason, P. K., Robinson, D. & Winchester, B. G. (1972) *Biochem. Biophys. Res. Commun.* **49**, 579–593
- Davidson, S. J., Hughes, W. L. & Barnwell, A. (1971) *Exp. Cell Res.* **67**, 171–187
- Davis, B. J. (1964) *Ann. N.Y. Acad. Sci.* **121**, 404–427
- Deter, R. L. (1971) *J. Cell Biol.* **48**, 473–489
- Deter, R. L. (1975a) *Exp. Cell Res.* **94**, 122–126
- Deter, R. L. (1975b) *Exp. Cell Res.* **94**, 127–139
- Deter, R. L., Baudhuin, P. & de Duve, C. (1967) *J. Cell Biol.* **35**, C11
- Dewald, B. & Touster, O. (1973) *J. Biol. Chem.* **248**, 7223–7233
- Flodin, P. (1961) *J. Chromatogr.* **5**, 103–115
- Hocking, J. D., Jolly, R. D. & Batt, R. D. (1972) *Biochem. J.* **128**, 69–78
- Hunter, W. M. & Greenwood, F. C. (1962) *Nature (London)* **194**, 495–496
- Kaulen, H. D., Henning, R. & Stoffel, W. (1970) *Hoppe-Seyler's Z. Physiol. Chem.* **351**, 1555–1563
- Kihara, H., Porter, M. T. & Fluharty, A. L. (1973) *Birth Defects Orig. Artic. Ser.* **9**, 19–26
- Kreibich, G., Debey, P. & Sabatini, D. D. (1973) *J. Cell Biol.* **58**, 436–462
- Marinković, D. & Marinković, J. (1976) *Biochem. J.* **155**, 217–223
- Martin, R. G. & Ames, B. N. (1961) *J. Biol. Chem.* **236**, 1372–1379
- Morell, A. G., Gregoriadis, G., Scheinberg, I. H., Hickman, J. & Ashwell, G. (1971) *J. Biol. Chem.* **246**, 1461–1467
- Neufeld, E. F. (1973) *Birth Defects Orig. Artic. Ser.* **9**, 27–30
- Nordén, N. E., Lundblad, A., Öckerman, P. A. & Jolly, R. D. (1973) *FEBS Lett.* **35**, 209–212
- Öckerman, P. A. (1967) *Lancet* **ii**, 239–241
- Phillips, N. C., Robinson, D. & Winchester, B. G. (1974a) *Clin. Chim. Acta* **55**, 11–19
- Phillips, N. C., Robinson, D. & Winchester, D. B. (1974b) *Biochem. J.* **137**, 363–371
- Philpot, J. St. L. & Stanier, J. E. (1956) *Biochem. J.* **63**, 214–223
- Robinson, D., Phillips, N. C. & Winchester, B. (1975) *FEBS Lett.* **53**, 110–112
- Taylor, H. A., Thomas, G. H., Aylsworth, A., Stevenson, R. E. & Reynolds, L. W. (1975) *Clin. Chim. Acta* **59**, 93–99
- Tsay, G. C. & Dawson, G. (1975) *Biochem. Biophys. Res. Commun.* **63**, 807–814