Pure & Appl. Chem., Vol. 57, No. 4, pp. 547–556, 1985.
Printed in Great Britain.

Diagnostic potential of serum and urine glycosidases in acquired diseases

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Abstract - Glycosidases are generally glycoproteins from lysosomal origin that catalyze the hydrolysis of glycoproteins, glycolipids and glycosaminoglycans, as well as synthetic substrates. They accomplish a very important role (partially known) in physiological circumstances, and their dysfunction causes pathological processes, where they have been mainly studied in connection to the lysosomal storage diseases.
After a brief review on the biosynthesis and catabolism of glycosidases and the factors influencing their activity, the present review article summarizes our own results on some glycosidase activities (β-N-acetylhexosaminidase, α-L-fucosidase, α-D-galactosidase, β-D-galactosidase, α-D-glucosidase, β -D-glucosidase, α-D-mannosidase, β-D-glucuronidase and β -D-fucosidase) determined in sera from patients of several acquired diseases (diabetes, pancreatitis, hepatitis, cirrhosis, breast and gastric carcinomas, myocardial infarction and renal deficiencies). Related papers of other authors are also quoted. In addition, we discuss our own results on some glycosidase activities determined in sera from patients who ingested a toxic oil and those from the same patients in their convalescence period; these determinations are an example of glycosidases as a useful index which contributes to follow the course of a disease. The signification of these enzymes in some renal disorders is also discussed. Finally, the convenience of introducing some glycosidase determinations (specially β-N-acetylhexosaminidase) in the routine work of Clinical Enzymology is proposed.

INTRODUCTION

From very early times, man has taken advantage of the empirical use of several glycosidases. Biological transformation in milk, starch degradation in cereals, etc, were biochemical processes that primitive and later civilized human beings employed without knowing their mechanism. During the first half of the XIX century, several scientists (Kirchoff, Payen and Persoz, Robiquet) studied some chemical reactions implicated in such processes. Unfortunately, this promising research on glycosidases was later developed slowly for nearly 100 years.

At the middle of the fifties of the XX century a biochemical discovery would change this situation: the finding by De Duve of a new intracellular organelle containing hydrolases, the lysosome.

On the other hand, the Clinical Enzymology was also a small chapter of the General Biochemistry in our century until the fifties. Phosphatases, lipases and amylases were the enzymes whose activities were more commonly determined with a diagnostic finality.

Immediately after the end of the second world war scientists paid a special attention to the possibility of enlarging the number of enzyme determinations which could be useful in that view. Furthermore, some artificial substrates were introduced to replace several natural ones, searching for easier and more precise determinations (performed generally by spectrophotometry)of the products released by the enzyme activity. So, progress in the enzyme techniques improved this branch of the Clinical Analysis and "Medicine could arrive, after the tissular and cellular periods, to the border of the Molecular Pathology", as it was written in 1971 (Ref. 1).

Later, a new area has emerged; i.e., the determination of metabolites of high interest (glucose, uric acid, etc) by using analytical reagents containing enzymes. Enzymes are employed as analytical tools, due to their specificity and efficiency. At present,the number of enzymes employed with this finality is gradually increasing.

In contrast, it might be asked why only about twenty enzymes (among the about 2,000 characterized enzymes) are generally determined for diagnostic of some acquired diseases. Perhaps the explanations to this fact are:(i) that these twenty enzymes are very well introduced both in the routine work of clinical laboratories and in the common knowledge of practioners; and (ii) that they give a satisfactory solution for the main diagnostic problems of these diseases.

However, it seems that Enzymology can enlarge this field by searching on other enzymes such as glycosidases, taking into account the characteristics of these enzymes.

The aim of this review is to summarize only the main results concerning the glycosidases whose activity has been determined in sera or urine from patients with some acquired disease.

It seems interesting to comment briefly some features about composition, biosynthesis and catabolism of the glycosidases, to justify the use of such materials for these assays as well as the advantages and limitations of these relatively new determinations.

GLYCOSIDASES AS GLYCOPROTEINS, AND CHARACTERISTICS OF GLYCOSIDASES

As it has been recently written (2), "glycosidases or carbohydrases are enzymes, included in class 3 of the Enzyme general clasification (IUB, 1979) that catalyze the hydrolysis of glycoconjugates (glycoproteins, glycolipids and glycosaminoglycans) as well as synthetic substrates which contain glycosidic linkages" (...). "Some of the reasons that may explain the emergence of a new knowledge on glycosidases after the decade of 1950, are the following: (a) the large distribution of these enzymes in Nature (actually considered as ubiquitous), which indicate their relevant role; (b) the demonstration of the occurence of glycosidases mainly in lysosomes, but also in other subcellular fractions; (c) the fact that an exoglycosidase from Escherichia coli, the β-galactosidase, has been employed in Molecular Biology, for informationists studies, as well as the lysozyme has been investigated in Molecular Biology for the structuralist approach; (d) the use of several glycosidases, either partially or completely purified, as tools for the determination of glycoconjugates structures; (e) the characterization of inherited metabolic disorders in which there is deficiency either of one glycosidase or one glycosidase isoenzyme; (f) finally, the recent knowledge on the role of some monosaccharides in the half-life of the plasma glycoproteins, regulated by the liberation of these carbohydrates by some glycosidases".

Glycosidases are generally glycoproteins (except lysozyme) and very often sialoglycoproteins. Several new reviews have appeared on glycoproteins (3-5) substances whose knowledge has been improved dramatically during the last few years. Reviews on nature and properties of glycosidases have algo been published (6, 7).

Many glycosidases exhibit multiplicity of forms, either from genetic origin (true isoenzymes) or from nongenetic origin (see later).

Although glycosidases have acidic pH optima, generally near 4.5 ("acid" glycosidases), some of them have a pH optimum near the neutrality (for instance about 6.5) when they show several forms.

They exhibit a wide specificity on the substrates.

Their main subcellular location is in lysosomes, however, they are found in other intracellular and extracellular compartments.

A. Lysosomes and biosynthesis of glycosidases

There are several glycosidases which are located not only in lysosomes but also in other locations; for example, α-D-mannosidase, in lysosomes, Golgi apparatus and cytosol; β-D-glucuronidase in lysosomes and endoplasmic reticulum, at least.

From the earliest reports of De Duve (8) and coworkers on the role of lysosomes on cell pathology, a large number of publications have appeared on this matter which have been summarized in several reviews (9-13). Nevertheless, De Duve recognized in 1983, in the 15th FEBS Meeting held at Brussels, that some topics concerning lysosomes are not completely understood yet.

Other important papers concerning the biosynthesis of lysosomal enzymes (14-16) and the role of oligosaccharides in glycoproteins (17-19) have been reviewed in the last four years.

B. Receptor-mediated endocytosis and clearance of glycosidases

After the classical work of Ashell and Morell (20), derived from a fortuitous observation appreciated by them in 1968, it has been established that for many plasma glycoproteins sialic acid is essential for their continued viability in the circulation, since on treating the glycoprotein with sialidase "galactose is exposed as the terminal nonreducing sugar of the protein-linked carbohydrate chains and serves as a specific determinant for the hepatic recognition of the sialic-deficient molecules. Conversely, the presence of intact sialic acid residues on the receptor sites of the hepatic plasma membrane is an absolute requirement for the initial binding reaction, wich in turn is a prelude to macromolecular transport and lysosomal catabolism" (20).

The purified rabbit liver receptor is also a "glycoprotein in which 10% of the dry weight consisted of sialic acid, galactose, mannose and glucosamine. The presence of calcium and the integrity of the terminal sialic acid residue were shown to be absolute requirements for binding" (21).

As Ashwell and col. have summarized in 1981 (22) and 1982 (23), "since the original discovery of the hepatic receptor for galactose-termined proteins, at least four alternate recognition systems have been described that are dependent on the exposure of specific sugars. These include the mannose-N-acetilglycosamine receptor in the reticuloendotelial system, the fucose receptor in mammalian hepatocytes, the N-acetylglucosamine receptor in avian liver, and the mannose-6-phosphate receptor in liver and fibroplasts. The latter system has assumed particular importance in that it appears to regulate the appropriate localization of the acid glycosidases within the lysosome" (22).

Although a relatively high number of papers (24-44) and review articles (23, 45-53) have been published on the clearance of lysosomal glycosidases and the role of receptors in these processes, some different points of view can be maintained on these topics. A cross situation for α-D-mannosidase (54) and α-D-glucosidase should be outlined since both enzymes participate in both biosynthesis and catabolism of glycoproteins.

It seems that glycosidases turnover is heterogenous since the measured degradation rates of the different individual enzymes are different; on the other hand, no correlation between half-life and molecular size has been found; furthermore, protein and carbohydrate moiety turn over as a unit, being the turnover relatively slow (55).

The constitutive and triggered lysosomal enzyme secretion (56), as well as the influence of exogenous amines on endocytosis, has also been recently reviewed (57).

C. Lysosomal enzyme precursors, activator proteins and protective factors

Works of Hasilik, von Figura, Neufeld and others have demonstrated that several hydrolases such as β-N-acetylhexosaminidase, α-N-acetylhexosaminidase, β -galactosidase, β-glucuronidase, α-glucosidase and cathepsin D are synthesized as precursors. The activation of the precursor is correlated by a reduction in its size in the case of cathepsin D; however, the precursor of β -N-acetylhexosaminidase A is able to hydrolyse GM_2 ganglioside on the presence of an activator (58).

In 1973, Li and coworkers (59) observed that crude β-N-acetylglucosaminidase A from human liver hydrolyzed glycophingolipids better than its purified preparations; this fact was due to the participation of an activator, identified by Li *et al.* in 1974 as a protein of 21 K-Da. Li and Li (59) concluded that human liver contains two different activators, one for GM_1 ganglioside degradation and other for GM_2 degradation. Other authors have also studied other activators from other organs (spleen, brain) which activate other gly-

cosidases (60).

Conzelmann et al. (61) have demonstrated that the activator protein extracts glycolipid monomers from micelles to give water-soluble complexes, being able in the absence of enzyme of transporting glycolipids from donor to acceptor membranes. So, deficiencies in hydrolases or in activator proteins could produce lipid storage disorders (62). On the other hand, it has been recently concluded that the carbohydrate moiety of some glycoproteins such as fibronectin plays an important role in stabilization or protection of the protein components against proteolysis (63).

Furthermore, the combined deficiency of β-galactosidase and neuraminidase in human fibroblasts is caused by the genetic deficiency of a 32 K-Da "protective protein" (which is also a glycoprotein); the addition of this factor can correct the excesive intralysosomal catabolism of the lysosomal β-galactosidase (64,65). It seems that this intralysosomal protection is carried out by aggregation of multimeric β-galactosidase, neuraminidase and protective factor (66).

Precursors of lysosomal enzymes, proteins which collaborate in their activity (activators) and glycoproteins which protect or stabilize them -as well as receptors for targetting, and targetting-ligands of the lysosomal enzymes- are probably efficient factors modulating the function of these enzymes by different and probably complementary mechanisms. Therefore the deficiencies in someone of these mechanisms will have an influence on the enzyme activity determined in any biological material.

CIRCUMSTANCES AND FACTORS INFLUENCING THE ACTIVITY OF LYSOSOMAL ENZYMES IN BIOLOGICAL FLUIDS

The group of lysosomal storage diseases will not be the matter of the present study. Several reviews on this subject have been published (3, 67-69). Let us only deduce that in the lysosomal storage diseases one or more lysosomal enzymes, or isoenzymes or activator or protective factors, are deficient or absent. Furthermore, it seems that the dysfunction arises rather from an accelered rate of degradation than from an alteration in its synthesis and transport, in several storage diseases, according to Jessup et al. (55).

Since glycoproteins are mainly degraded by hydrolases present in the lysosomes inside the cell, and glycosidases and proteases occur at higher concentration gradients intracellularly respective to extracellularly (see later), the rise of these enzyme activities in biological fluids such as serum, plasma or urine is a not well understood matter, although it is, paradoxically, the basis supporting a great number of enzyme determinations accomplished in these fluids with a diagnostic finality.

Both points require some discussion:

Lysosomal enzymes are found into the cell at high levels, and normally at low levels in the plasma and other fluids where they come after exocytosis from the cell. In addition, enzyme activity is measured in the biological fluids, not enzyme concentration. The levels of this activity in such fluids reflect the following balance:

Enzyme biosynthesis (+ Activation for drugs,etc) ⇌

⇌ Enzyme degradation (+ Inactivation for drugs, poisons,enzyme "ageing", etc.)

(The low volume of samples and their dilution generally reduce the influence of drugs, etc, as activators or inhibitors in the determination of enzyme activity).

Alterations in this normal balance, with a rise in the levels of enzyme in extracellular fluids, could be the result of (I) pathological circumstances. For instance:

1. A cellular proliferation, with or without modification of the membrane permeability.
2. An increased permeability of the citoplasma and/or lysosomal membranes, the cell number being normal.

Several agents or situations can modify the citoplasmic membrane giving either necrose or light changes in the permeability:

(a) Bacterial toxins or infective agents.
(b) Chemical compounds, poisons such carbon tetrachloride or drugs.
(c) Deficiencies in oxygen supply, depletion of circulating glucose or agents which block any metabolic pathway needed for ATP biosynthesis (since ATP is required for the membrane functions).

Other authors (70, 71) have also summarized some interpretations on the mechanisms of hydrolases leakage.

II. In addition to those pathological circumstances, other, physiological circumstances, should be taken into consideration, such as:

1. The concentration gradient of the enzyme, which varies for the enzymes, but may be 3,000-50,000-fold higher inside the cell than extracellularly.
2. The size of the enzyme molecule, ranging from low to high values. (It explains that enzymes such as amilase, the mol. weight of which is relatively low, 45,000, can be eas ly excreted by urine).
3. The intracellular location of the enzyme.

Briefly, enzymes at a high concentration gradient in the cell, or not associated to membranes or with low mol. weight can pass through the semi-permeable barrier which is the citoplasmic membrane better than others, and be finally found in the plasma at higher concentrations than others. In addition, other factors or circumstances (such as the turnover) influence those levels.

Finally, general criteria to select enzymes with analytical application could be the specificity, sensitivity and reproducibility of the techniques employed, and the period of apparition and length of the modifications of the enzyme activity for one metabolic disorder.

RESULTS OBTAINED ON SEVERAL GLYCOSIDADES IN SERUM AND URINE FROM PATIENTS OF SOME ACQUIRED DISEASES

Although lysosomal enzymes play an important role in clinical research and lysosomes were discovered at the middle of the fifties, the number of papers on this area was relatively low until the eighties. The availability of new commercial substrates has probably encouraged these investigations. At present, the colorimetric assays using substrates such as p-nitrophenyl glycosides have been replaced by the fluorimetric ones employing methylumbelliferyl glycosides because of the generally higher sensitivity of the fluorimetric techniques relative to the spectrophotometric determinations.

TABLE 1. Glycosidase activities in sera from patients of some acquired diseases.

	Diabetes mellitus	Acute pancreat.	Acute viral hepatit.	Hepatic cirrhos.	Gastric carcin.	Breast cancer	Acute myoc. infarction	Renal deficien.	Toxic illness
β-N-Acetylgluc.	118	173	224	164	120	136	133	68	170
β-N-Acetylgal.	119	n.d.	n.d.	187	129	n.d.	n.d.	99	n.d.
β-D-Glucur.	125	n.d.	n.d.	142	84	n.d.	n.d.	104	145
α-L-Fucosid.	504	123	201	577	699	105	114	275	156
β-D-Glucos.	n.d.	159	188	n.d.	n.d.	153	128	n.d.	400
α-D-Galact.	122	n.d.	n.d.	144	160	n.d.	n.d.	93	66
β-D-Galact.	126	88	100	144	157	89	61	90	159
α-D-Glucos.	n.d.	128	200	n.d.	n.d.	129	142	n.d.	83
α-D-Mannos.	115	n.d.	n.d.	107	112	n.d.	n.d.	89	134
β-D-Fucosid.	n.d.	92	167	n.d.	n.d.	122	133	n.d.	n.d.

The value 100 is arbitrary assigned to normal values; n.d. = not determined.
Generally, the number of samples, n, was 10; exceptionally, for patients of toxic illness n=17 in control groups, and n=50-113 in experimental groups.
Values underlined as - -- are lower than controls.
In values underlined as —— the difference was highly significant ($p<0.001$).

A. Results concerning serum glycosidases

Table 1 summarizes, comparatively, some results of our department (72-74) on glycosidases activities determined in sera from patients with diabetes mellitus, acute pancreatis, acute viral hepatitis, hepatic cirrhosis, gastric carcinoma, breast cancer, acute myocardial infarction and renal deficiencies.

The values are significantly higher in sera from patients, relative to controls, for many enzyme activities; however, there are also some activities which are significantly lower than controls; and other, finally, which are similar in both groups. Table 1 also shows that some activities such as β-N-acetylglucosaminidase, β-D-glucuronidase, α-L-fucosidase and β-D-glucosidase are significantly increased in patients who ingested a toxic oil (acquired without sanitary authorization) respective to controls (75,76). In contrast, α-D-galactosidase and β-D-galactosidase exhibit values significantly lower in patients than in controls and α-D-glucosidase and α-D-mannosidase show similar values in both groups.

The data of Table 1 suggest the following questions:

1. Why are some of the above mentioned glycosidase activities of the experimental groups increased, other disminished, and other similar in both groups?

2. Why are enzyme activities such as β-N-acetylhexominidase or β-D-glucosidase or α-L-fucosidase generally increased in very different disorders, while other such as β-D-galactosidase or α-D-galactosidase are disminished, at least in some of them?

We have not a definitive answer to these questions. However, all factors discussed before could help us to find a partial interpretation to these facts. Thus, one alteration in the cell membrane (produced by very different agents or circumstances) could be responsible for the release of intralysosomal enzymes into the blood; this release could be accomplished for some enzymes more easily than for others depending on their mol. weight and subcellular location. In addition, other interpretations such as those referring to modifications in the receptors, alteration in the turnover of the enzymes, etc, are not precluded.

In connection with this matter, Stahl and coworkers (77) have found that the injection of organophosphates to rats elicits a massive increase in plasma β -glucuronidase, correlated with a fall in its microsomal activity, and independent of protein synthesis; nevertheless, the plasma activity of other lysosomal enzymes remains unchanged; these autors suggest that microsomal β -glucuronidase must be modified before secretion by a post-translational mechanism.

Results similar to ours have been generally found also by other authors (78,79) for glycosidases in patients with leukemia (78) and Grave's disease (79).

In addition, the results concerning other disorders for β-N-acetylhexosaminidase, which is the serum (or plasma) enzyme with the highest activity of the glycosidases commonly determined, are also in agreement with ours; so, in diabetes, myocardial infarction and liver diseases (80), varicous states (81), diabetes (82-84) and liver disorders (85,86).

From a few years, the studies on glycosidase isoenzymes (see reviews 87-89) have demonstrated to be of great interest. Reviews (90) concerning β-N-acetylhexosaminidase isoenzymes and papers (91-95) on their properties and determination have appeared. Probably in a next future, the measure of total activity of some glycosidases should be accompanied by that of their isoenzymes in view of getting a better information on many lysosomal diseases.

α -L-Fucosidase is another glycosidase whose levels are relatively high in several disorders. Precisely a paper on the advantages of the fluorimetric determination of this enzyme and its forms was published as early as 1974 by Robinson and Thorpe (96). The presence of low and high activity forms of α -L-fucosidase in normal human serum is a peculiarity of this enzyme which has been studied by some authors (94-103). Its substrate specificity (104) and levels in diabetics (105) have also been studied.

Results on α-D-mannosidase (105,106), α-D-galactosidase (105,107), β-D-galactosidase (107), α-D-glucosidase, β-D-glucosidase and β-D-glucuronidase activities in plasma (or serum) are less common, and have generally been

published together with those of β-N-acetylhexosaminidase.

β -D-Fucosidase is a special activity corresponding to a well characterized enzyme (2) which shows a wide variability in its properties concerning the association to other activities (β -D-galactosidase, β-D-glucosidase); however, their values in sera from patients of some illness do not seem to be paired with those of β-D-galactosidase and β-D-glucosidase (see Table 1).

B. Usefulness of those determination for following the course and detection of some diseases.

There are few publications on glycosidases as a measure for following the course of a disease. We have determined the activity of glycosidases in sera from 21 convalescent patients who ingested a toxic oil, taking the blood samples on 3 or 4 occasions during a period of 11-12 months (108), and pursuing a previous study in which the same patients were at the acute phase of their illness (75, 76). The enzyme activities which were higher than controls during the first 1-3 months of the study decreased after few months and became normal, while those which were initially lower, increased and finally were similar (or higher) to controls, being all these results correlated with the improvement in the general health state of the patients (108).

Another important topic is that concerning the eventual use of some glycosidase determinations in preventive medicine for detection of pathological situations not declared by typical symptoms. The rapport of Tettamanti and coworkers (107) is particulary illustrative on the study of workers exposed to inorganic mercury vapours as well as on the assay and significance of enzymes of lysosomal origin in plasma (or serum).

C. Results concerning urine glycosidases

In some cases, the general characteristics of urine reduce its use for enzyme analysis. However, the determination of glycosidases (mainly β-N-acetylhexominidase and its isoenzymes) in urine is very interesting in the research of renal disorders or after renal transplantation or for following the nephrotoxicity caused by amino glycosidic antibiotics. Besides, it can be performed by automatic procedures (109).

One excellent review on urinary enzymes, nephrotoxicity and renal diseases is that of Price (110). Other recent papers relative to these topics are: On the use of ω-nitrostyryl substrates for colorimetric assays in urine (111), glycosidases in hypertension (112), in urinary tract infections (113), in diabetes (114), after renal transplantation (115, 116), in multiple myeloma (117) and for evaluating nephrotoxicity of aminoglycosides or other substances (118-120).

D. Practical suggestions

1. The comparison of the results from different (or from the same) laboratories is very often a difficult task due to the use of different analytical procedures or assay conditions. In addition, the expression of the enzyme activity values is not uniform. Thus, it would be very convenient to normalize the experimental protocols for these enzymes. Actually the detailed description of some common techniques, for example those concerning β-N-acetylhexosaminidase (121) and α-L-fucosidase (122), are easy to find.

2. The stability of glycosidases seems to be generally higher in plasma than in serum, according to Lombardo et al. (123); in contrast, the kind of anticoagulant does not seem to influence the plasma activity values (123). Therefore Lombardo and coworkers propose to employ plasma and not serum for these analysis and to perform the assays "with freshly prepared plasma no later than 1 h after its preparation" (124).

3. In addition, sex has no influence on the plasma values of β-N-acetylhexosaminidase, β-D-galactosidase, α-L-fucosidase and α-D-mannosidase (although it does in β -D-glucuronidase values, which are about 30% higher in males) (124); but age does, and an activity enhancement has been observed in people around 30-40 years old (124).

4. Furtheremore, the use of substrates at the convenient concentrations (124) and, in some cases, to check them (if they are artificial) against the natural ones to avoid confusions (125, 126) should also be recommended.

CONCLUSIONS

1. A great progress has been achieved during approximately the last ten years in the knowledge on plasma, serum and urine glycosidases related to some acquired diseases; but further research is required to develop this knowledge and to avoid limitations such as those caused by overlapping of some experimental and control results, etc.

2. However, from the data discussed above, it seems that the determination of some glycosidase activities (mainly β-N-acetylhexosaminidase) and their isoenzymes in plasma (or serum), and urine (for the study of renal disorders), is interesting and should be performed more frequently, at least in some research laboratories in which other enzyme activities are measured with a clinical finality, as a previous step to generalize their use in the routinary work of Clinical Enzymology.

Acknowledgements - The results concerning the works of our department included in this study were obtained in collaboration with the authors indicated in the references list: Prof. Angel Reglero, Prof. Pedro Calvo, Miss Isabel Carretero (Graduate in Pharmacy), Mr. José L. Barba (Graduate in Pharmacy) and Mr. Manuel J. Cabezas (Graduate in Medicine); their kind collaboration is acknowledged. The autor wishes to thank the Organizing Committee of the Congress for the invitation, Prof. Pedro Calvo for the critical reading of the manuscript, and Miss Belén for the typing.

This work was supported by the Ministerio de Educación and by the C.S.I.C.

REFERENCES

1. P. Boivin, A. Gajdos and H. Lestradet, Enzymopathies, p.v., Masson, Paris (1971).
2. J. A. Cabezas, A. Reglero and P. Calvo, Int. J. Biochem. 15, 243-259 (1983).
3. J. Montreuil, Glycoproteins (Comprenhensive Biochemistry, vol. 19, pp. 1-188, Elsevier, Amsterdam (1982).
4. R.C. Hughes, Glycoproteins, pp. 1-95, Chapman and Hall, London (1983).
5. N. Sharon, Trends Biochem. Sci. 9, 198-202 (1984).
6. J.M.W. Bouma, in Enzyme Therapy in Lysosomal Storage Diseases, pp. 197-206, North-Holland, Amsterdam (1974).
7. H.M. Flowers and N. Sharon, Advanc. Enzymol. 48, 29-95 (1979).
8. C. De Duve, Triángulo 9, 200-208 (1959).
9. A.B. Novikoff, Science, 8, 518-519 (1974).
10. K. Aterman, Histochem. J. 11, 503-541 (1979).
11. O. Touster, Mol. Cell. Biochem. 2, 169-177 (1973).
12. L.D. Strawser and O. Touster, Rev. Physiol. Biochem. Pharmacol. 87, 169-210 (1980
13. J.M. Tager, P.G. De Groot, M.N. Hamers, M. Hollemans, R.Kalsbeek, A. Strijland and F.P.W. Tegalaers, Cell Biological Aspects of Disease, pp. 235-250, Martinus Nijhoff, The Haghe (1981).
14. A. Hasilik, Trends Biochem. Sci. 5, 237-240 (1980).
15. M. Imort, M. Zuhlsorf, U. Reige, A. Hasilik and K. Figura, Biochem J. 214, 671-78 (1983).
16. R.T. Schwarz and R. Datema, Trends Biochem. Sci. 9, 32-34 (1984).
17. R. Gibson, S. Kornfeld and S. Schlesinger, Trends Biochem. Sci.5, 290-293 (1980).
18. R.C. Hughes and T.D. Butters, Trends Biochem. Sci. 6, 228-230 (1981).
19. S.A. Barker and C.J. Gray, Biochem. Soc. Trans. 11, 16-17 (1983).
20. G. Ashwell and A.G. Morell, Advanc. Enzymol. 41, 99-128 (1974).
21. G. Ashwell and A. Morell, Trends Biochem. Sci. 2, 76-78 (1977).
22. G. Ashwell and C.J. Steer, J. Am Med. Assoc. 246, 2358-2364 (1981).
23. J. Harford and G. Ashwell, in The Glycoconjugates, pp. 27-55, Academic Press, New York (1982).
24. P. Stahl, B. Mandell, J.S. Rodman, P. Schlesinger and S. Lang, Arch. Biochem. Biophys. 170, 536-546 (1975).
25. P. Stahl, H. Six, J.S. Rodman, P. Schlesinger, D.R.P. Tulsiani and O. Touster, Proc.Natl. Acad. Sci. USA 11, 4045-4049 (1976).
26. P. Stahl, J.S. Rodman and P. Schlesinger, Arch. Biochem. Biophys., 117, 594--605 (1976).
27. P. Schlesinger, J.S. Rodman, M.Frey, S.Lang and P. Stahl, Arch.Biochem. Biophys. 177, 606-614 (1976).
28. P.D. Stahl, J. S. Rodman, M.J. Miller and P.H. Schlesinger, Proc. Natl. Acad. Sci. USA 75, 1399-1403 (1978).
29. P.H. Schlesinger, T.W. Doebber, B.R. Mandell, R. White, C. Deschryver, J.S. Rodman, M.J. Miller and P. Stahl, Biochem. J. 176, 103-109 (1978).
30. K. Ullrich, V. Gieselmann, G. Mersmann and K. Von Figura, Biochem. J. 182-329-335 (1979).

31. C.J. Steer, J.W. Kusiak, R.O, Brady and E.A. Jones, Proc. Natl. Acad. Sci. USA 76, 2774-2778 (1979).
32. C.J. Steer and G. Ashwell, Jour. Biol. Chem. 255, 3008-3013, (1980).
33. H. Tolleshaug and T. Berg, Hoppe-Seyler's Physiol. Chem. 361, 1155-1164 (1980).
34. J. Mizuno, Y. Kozutsumi, T. Kawasaki and I. Yamashina, J. Biol. Chem. 256, 4247-4252 (1981).
35. A. L. Schwatz, S. E. Fridovich, B.B. Knowles and H.F. Lodish, J. Biol. Chem. 256, 8878-8881 (1981).
36. M.D. Skudlarek and R.T. Swank, J. Biol. Chem. 256, 10137-10144 (1981).
37. W. Jessup and R.T. Dean, Biochem. Biophys. Res. Commun. 105, 922-927 (1982).
38. Bridges, K, J. Harford, G. Ashwell and R.D. Klausner, Proc. Natl. Acad. Sci. USA 79, 350-354 (1982).
39. J. U. Baenziger and D. Fiete, J. Biol. Chem. 257, 6007-6009 (1982).
40. C. A. Gabel, D.E. Goldberg and S. Kornfeld, Proc. Natl. Acad. Sci. USA 80, 775--779 (1983).
41. R. Tauber, C.S. Park and W. Reutter, Proc. Natl. Acad. Sci. USA 80, 4026--4029 (1983).
42. A.L. Schwartz and D. Rup, J. Biol. Chem. 258, 11249 11255 (1983).
43. J.L. Bodmer and R.T. Dean, Biochem. Biophys. Res. Commun. 113, 192-198 (1983).
44. R. Pohlmann, S. Krüger, A. Hasilik and K. V. Figura, Biochem. J. 217, 649-658 (1984).
45. P.D. Stahl and P.H. Schlesinger, Trends Biochem. Sci. 5, 194-196 (1980).
46. J.G. McFarlane, Clin. Sci. 64, 127-135 (1983).
47. W. Gly, in The Glycoconjugates, pp. 3-25, Academic Press, New York (1982).
48. A. Helenius, I. Mellman, D. Wall and A. Hubbard, Trends Biochem. Sci. 8, 245-250 (1983).
49. I. Patan and M.C. Willingham, Trends Biochem. Sci. 8, 250-254.
50. J.M. Besterman and R.B. Low, Biochem. J. 210, 1-3 (1983).
51. J.B. Lloyd and K.E. Williams, Biochem. Soc. Trans. 12, 527-528 (1984).
52. A. Dautry-Varsat and H.F. Lodish, Invest. Ciencia 94, 34-41 (1984).
53. M. F. Dean and S. Diment, Biochem. Soc. Trans. 12, 524-526 (1984).
54. B. Winchester, Biochem. Soc. Trans. 12, 522 (1984).
55. W. Jessup, J.L. Bodmer, R.T. Dean. V.A. Greenaway and P. Leoni, Biochem. Soc. Trans. 12, 529-531 (1984).
56. W. Jessup, P. Leoni, R.T. Dean, in Developments in Cell Biology, vol. 1, in press, Butterworths London (1984).
57. R. T. Dean, W. Jessup and C.R. Roberts, Biochem J. 217, 27-40 (1984).
58. A. Hasilik, K. V. Figura, E. Conzelmann, H. Nehrkorn and K. Sandhoff, Eur. J. Biochem. 125, 317-321 (1982).
59. Y.T. Li and S.C. Li, in Lysosomes and Lysosomal Storage Diseases, pp. 173-180, Raven Press, New York (1981).
60. K. Inui and D.A. Wenger, Biochem. Biophys. Res. Commun. 105, 745-751, 1982.
61. E. Conzelmann, J. Burg, G. Stephan and K. Sandhoff, Eur. J. Biochem. 123, 455--464 (1982).
62. K. Sandhoff and E. Conzelmann, Trends Biochem. Sci. 4, 231-233 (1979).
63. B.A. Bernard, K.M. Yamada and K. Olden, J. Biol. Chem. 257, 8549-8554 (1982).
64. A. Hoogeveen, A. D'azzo, R. Brossmer and H. Galjaard, Biochem. Biophys. Res. Commun. 103, 292-300 (1981).
65. A. D'Azzo, A. Hoogeveen, A.J.J. Reuser, D. Robinson and H. Galjaard, Proc. Natl. Acad. Sci. USA 79, 4535-4539 (1982).
66. A.T. Hoogeveen, F.W. Verheijen and H. Galjaard, J. Biol. Chem. 258, 12143-1246 (1983).
67. G. Strecker and J. Montreuil, Biochimie 61, 11-12 (1979).
68. J. Montreuil, Compt. Rend. seanc. Soc. Biol. 175, 694-709 (1981).
69. K. V. Figura and A. Hasilik, Trends. Biochem. Sci. 9, 29-31 (1984).
70. B.L. Reed, Aust. J. Hosp. Pharm. 8, 87-90 (1978).
71. N. F. LaRusso, Digest. Dis. Scienc. 24, 177-179 (1979).
72. A. Reglero, M. I. Carretero and J.A. Cabezas, Clin. Chim. Acta 103, 155-158 (1980).
73. P. Calvo, J.L. Barba and J. A. Cabezas , Clin. Chim. Acta 119, 15-19 (1982).
74. A. Reglero Chillon, M.I. Carretero Casimiro and J.A. Cabezas Fdez. del Campo, An. Real Acad. Farm. 46, 55-68 (1980).
75. M.J. Cabezas-Delamare, A. Reglero and J.A. Cabezas, Clin. Chim. Acta 128, 53--59 (1983).
76. M. J. Cabezas-Delamare, A. Reglero and J.A. Cabezas in Programa del CSIC para el estudio del síndrome tóxico, pp. 477-487, C.S.I. C., Madrid (1983).
77. B. Brian Mandell and P. Stahl, Biochem. J. 164, 549-556 (1977).
78. T. Tanaka, M. Kobayashi, O. Saito, N. Kamada, A. Kuramoto and T. Usui, Clin. Chim. Acta 117, 121-131 (1982).
79. H. Guillou, V. David, Y. Lorcy, A. Le Treut, H. Allannic and J.Y. Le Gall, Clin. Chim. Acta 120, 219-224 (1982).
80. J.W. Woolen and P. Turner, Clin. Chim. Acta 12, 671-683 (1965).
81. P. Niebes, Clin. Chim. Acta 42, 399-408 (1972).
82. N.J. Van Haeringen, J.I. Vrooland and E. Glasius, Clin. Chim. Acta, 86, 333-338 (1978).
83. J.A. Alhadeff and R.T. Holzinger, Biochem. Med. 27, 214-225 (1982).
84 J.M. Miralles, J.J. Corrales, L.C. García-Díez, J.A. Cabezas and A. Reglero, Clin. Chim. Acta 121, 373-378 (1982).

85. A.M. Gressner and P. Roebruck, Clin. Chim. Acta, 124, 315-326 (1982).
86. B. Joelsson, B. Hultberg, A. Isaksson, A. Alwmark, P. Gullstrand, Clin. Chim. Acta 136, 203-209 (1984).
87. Robinson D., in Enzyme Therapy in Lysosomal Storage Diseases, pp. 217-226, North-Holland, Amsterdam (1974).
88. J.A. Kint, D. Carton, A. Huys and C. Hooft in Enzyme Therapy in Lysosomal Storage Diseases, pp. 227-237, North-Holland, Amsterdam (1974).
89. J.C. Dreyfus and Poenaru, L. in Enzyme Therapy in Lysosomal Storage Diseases, pp. 259-263, North-Holland, Amsterdam (1974).
90. J.A. Lowden, D. Mahuran, A. Novak, C. Lee and M.A. Skomorowski, Lysosomes and Lysosomal Storages Diseases, pp. 181-193, Raven Press, New York (1981).
91. M. Drogin, S. Nakagawa and H.M. Nitowsky, J. Biochem. 8, 849-856 (1977).
92. S.M. Tucker, R. J. Pierce and R. G. Price, Clin. Chim. Acta 102, 29-40 (1980).
93. T. Momoi, K. Kikuchi, Y. Shigematsu, M. Sudo and K. Tanioka, Clin. Chim. Acta 133, 331-334 (1983).
94. W. Fuchs, R. Navon, M.M. Kaback and H. Kresse, Clin. Chim. Acta 133, 253-261 (1983).
95. A. Orlacchio, C. Maffei, T. Beccari and P. Lisi, Clin. Chim. Acta 141, 127-133 (1984).
96. D. Robinson and R. Thorpe, Clin. Chim. Acta 55, 65-69 (1974).
97. S. Wood, J. Lab. Clin. Med. 88, 469-476 (1976).
98. J.A. Alhadeff and J.S. O'Brien, in Practical Enzymology of the Sphingolipidoses, pp. 247-281, Alan R. Liss, New York (1977).
99. J.A. Alhadeff and G.L. Andrews-Smith, Biochim. Biophys. Acta 614, 466-475 (1980).
100. J.A. Alhadeff in Lysosomes and Lysosomal Storages Diseases, pp. 299-314, Raven Press, New York (1981).
101. M. Bernard, M.J. Foglietti and F. Percheron, Clin. Chim. Acta 116, 91-99(1981).
102. M. Bernard, F. Percheron and M.J. Foglietti, Int. J. Biochem. 15 1385-1387 (1983).
103. P. J. Willems, E. Romeo, W.R. Den Tandt, A.F. Van Elsen and J.G. Leroy, Human Gen. 53, 115-118 (1982).
104. R.A. DiCioccio, J.J. Barlow and K.L. Matta, J. Biol. Chem. 257, 714-718 (1982).
105. J.A. Alhadeff, R.T. Holzinger and G.L. Andrews-Smith, Biochem. Med. 28, 115-121 (1982).
106. J. Butterworth, Clin. Chim. Acta 108, 347-353 (1980).
107. G. Tettamanti, A. Lombardo, L. Caimi and V. Foá in Clinical Enzymology Symposia, pp. 59-75, Picin Medical Books, Padua (1977).
108. M.J. Cabezas-Delamare and J. Cabezas, Clin. Chim. Acta (in press).
109. S.M. Tucker, P.J.R. Boyd, A.E. Thompson and R.G. Price, Clin. Chim. Acta 62 333-339 (1975).
110. R.G. Price, Toxicology, 23, 99-134 (1982).
111. C.T. Yuen, R.G. Price, L. Chattagoon, A.C. Richardson and P.F.G. Praill, Clin. Chim. Acta 124, 195-204 (1982).
112. I.D.A. Johnston, N.F. Jones, J.E. Scoble, C.T. Yuen and R.G. Price, Clin. Chim. Acta 133, 317-325 (1983).
113. A. Vigano, B.M. Assael, A.D. Villa, L. Gagliardi, N. Principi, P. Ghezzi and M. Salmona, Clin. Chim. Acta 130, 297-304 (1983).
114. B.H. Brouhard, L. LaGrone, L.B. Travis and T.G. Pollard, Clin. Chim. Acta 140 197-202 (1984).
115. P.R.N. Kind, Clin. Chim. Acta 119, 87-97 (1982).
116. P.H. Whiting, J. Petersen, D.A. Power, R.D.M. Stewart, G.R.D. Catto and N. Edward, Clin. Chim. Acta 130, 369-376 (1983).
117. R.A. Coward, I.W. DeLamore, N.P. Mallick and L.E. Robinson, Clin. Chim. Acta 138, 293-298 (1984).
118. U. Diener, E. Knoll, B. Langer, H. Rautenstrauch, D. Ratge and H. Wisser, Clin. Chim. Acta 112, 149-157 (1981).
119. R. Gibey, J.L. Dupond, D. Alber, R.L. des Floris and J. C. Henry, Clin. Chim. Acta 116, 25-34 (1981).
120. R. Gibey, J.L. Dupond and J.C. Henry, Clin. Chim. Acta 137, 1-11 (1984).
121. J.L. Stirling, in Methods Enzymatic Analysis, vol. IV, pp. 269-277, Verlag Chemie, Weinheim (1984).
122. G. Tettamanti, M. Masserini, G. Goi and A. Lombardo, in Methods Enzymatic Analysis, vol. IV, pp. 263-269, Verlag Chemie, Weinheim (1984).
123. A. Lombardo, L. Caimi, S. Marchesini, G. Carlo Goi and G. Tettamanti, Clin. Chim. Acta 108, 337-346 (1980).
124. A. Lombardo, G. Carlo Goi, S. Marchesini, L. Caimi, M. Moro and G. Tettamanti, Clin. Chim. Acta 113, 141-152 (1981).
125. S. Gatt, in Enzyme Therapy in Lysosomal Storage Diseases, pp. 206-215, North-Holland, Amsterdam (1974).
126. A.W. Schram and J. M. Tager, Trends Biochem. Sci. 6, 328-330 (1981).