*Carbohydrate Research*, 227 (1992) 339–349
Elsevier Science Publishers B.V., Amsterdam



# Molecular and metabolic aspects of lysosomal glycogen*

Robert Geddes†, Pooranalingham Jeyarathan, and Jacqueline A. Taylor
*Department of Biochemistry, University of Auckland, Auckland (New Zealand)*

(Received July 9th, 1991; accepted December 19th, 1991)

ABSTRACT

The high molecular weight glycogen associated with the lysosomal compartment in glycogen storage disease type VIII is more resistant to degradation by proteinase than normal glycogen. The assembly of large glycogen particles on disulphide-linked protein backbones has been confirmed and the disulphide-reducing nature of the lysosome appears to confer an advantage in the amylolytic degradation of glycogen.

Experiments utilising acarbose, a lysosomal (1→4)-α-D-glucosidase inhibitor, show that some blood glucose could arise in normal mammals from extra-hepatic tissue, by degradation of the glycogen in the lysosomal compartment.

## INTRODUCTION

Over many years, Manners and his co-workers[1–6] have reported on the structures of glycogens and their (often subtle) variations. The molecular weight distributions of glycogens isolated from different sources, or from one source under different circumstances (*e.g.*, starvation, post-mortem), indicated another level of complexity[7–12]. The proteoglucan nature of glycogens from liver and muscle and the relationship of disulphide bonding to high molecular weight molecules are now well established[13–19].

The cause of these variations lies in the complex and continually active synthesis, storage, and degradation of glycogen. Since the classic biochemical description of glycogenosis type II (GSDII) by Hers[20], a minimum of 10% of the tissue-glycogen store in liver and muscle has been found in the lysosomal compartment[10,21–24]. The lysosomal glycogen is in the high molecular weight range, which explains, at least partially, the size and polydispersity of glycogens, by providing a two-compartment metabolism model[22,25–30]. We have addressed three aspects of the structure and metabolism of lysosomal glycogen.

(*a*) Because of its proteoglucan nature, the high molecular weight glycogen found in lysosomes can be degraded by proteinases (*e.g.*, proteinase K) without any degradation of carbohydrate[17]. Likewise, there is little effect on the low molecular weight non-lysosomal (cytosolic) glycogen. Since animals that lack phosphorylase kinase (glycogenosis type VIII) (GSDVIII) have both a relative, and an absolute, excess of non-lysosomal glycogen[31] (but the lysosomal glycogen is not depleted on moderate

* Dedicated to Professor David Manners.
† Author for correspondence.

0008-6215/92/$ 05.00 

starvation[31]), proteinase K should have a similar effect to GSD type VIII *unless* there are additional induced structural changes unrelated to the carbohydrate portion of glycogen, in this genetic disorder.

(*b*) The interior of lysosomes is more acidic than the surrounding cytosol[32,33], and Lloyd[34] has postulated that lysosomes possess a disulphide-reducing capacity, linked to a cysteine pump. High molecular weight glycogen is converted, *in vitro*, by disulphide-bond breakers into its low molecular weight counterpart without any loss of carbohydrate[13]. Therefore, it was relevant to investigate whether disulphide-bond degradation confers any advantage on the amylolytic degradation of glycogen.

(*c*) Acarbose, an inhibitor of the lysosomal (1→4)-α-D-glucosidase, disrupts the metabolism of lysosomal glycogen in both normal and GSD type VIII animals, and also effects changes in normal animals that mimic GSD II[22,29–31]. Since lysosomal enzymes are acid hydrolases and may depend upon the levels of cytosolic lactate and pyruvate for their activity, these levels have been measured in normal and GSD VIII animals being starved and/or treated with acarbose. Since glucose is the product of the lysosomal degradation of glycogen, blood glucose levels were monitored in parallel.

## EXPERIMENTAL

Acarbose (Bay g 5421), a gift from Professor R. Lullman-Rauch (Department of Anatomy, University of Kiel, F.R.G.), was dissolved in physiological saline and administered by i.p. injection (400 mg/kg), and the rats were sacrificed either 24 h or 5 days after injection. Half of the animals were starved 17 h prior to sacrifice. Normal rats were of a random bred Wistar strain. Phosphorylase kinase-deficient animals (kindly supplied by Dr. R. S. Malthus and Professor J. G. T. Sneyd of the Department of Clinical Biochemistry, the University of Otago, New Zealand) were of the NZR/Mh strain homozygous for the enzymic deficiency (gsd/gsd)[35,36] (glycogen storage disease type VIII, GSD VIII). The animals were maintained under standard animal-house conditions (14 h light, 10 h dark, 20 ± 1°).

Glycogen was extracted from rat livers as described, with care being taken to minimise the rapid non-uniform degradation. Lysosomal glycogen was isolated from rapidly prepared lysosome-enriched subcellular fractions by a modified cold-water extraction method[21,22]. The reproducibility of the lysosomal preparations was enhanced significantly by the use of a constant-velocity automatic homogeniser of the type described by Dingle and Barrett[37]. Fractionation of glycogen and determination of the molecular weight were carried out as described[10,21].

Figs. 2 and 3 are presented as glycogen percentage versus fraction number rather than molecular weight, because of the better visual spread of the data. The fraction number may be converted into the approximate molecular weight by using Table IV in ref. 10.

Chain lengths and beta-amylolysis limits were determined by standard methods[15] using pullulanase and beta-amylase (Boehringer). The proteinase K sample (EC 3.4.21.14, Boehringer) had no significant saccharase activity. Glycogen solutions (1

mg/mL) in borate buffer (0.1M, pH 7.5) were incubated with 1 ng of the purified enzyme (37°, 6 h). The reaction was terminated by brief exposure to boiling water. Recovery of carbohydrate after treatment with proteinase K averaged 99%, and recovery of protein was in the range 7–13%. Protein content was measured by the method of Bradford[38].

For treatment with 2-mercaptoethanol, freeze-dried glycogen was dissolved (2 mg/mL) in 0.1M Tris buffer (pH 8.5) containing 8M urea. After bubbling oxygen-free nitrogen through the solution for 30 min, the sample was treated with 2-mercaptoethanol (0.1 mL/mL, 30 min), and half was then treated with iodoacetamide (0.3 g/mL). Both parts were then dialysed overnight against running water. Glycogen samples were exposed to alpha-amylase (*B. subtilis*, Koch–Light) solutions (0.1M Tris buffer) for various times.

## RESULTS AND DISCUSSION

*GSD VIII lysosomal glycogen.* — Table I contains data on the glycogens isolated from the livers of normal rats, and animals deficient in the cytosolic enzyme, phosphorylase kinase (GSD VIII). As found by others, there is enhanced storage of glycogen[36], but the protein content and structure of the glycogen do not vary significantly. Fig. 1

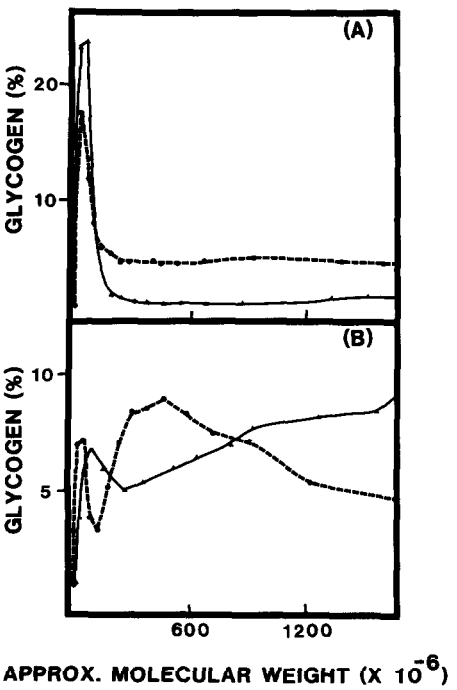


Fig. 1. Molecular weight distributions, measured after sucrose-density-gradient centrifugation, of glycogens isolated from livers of normal (--●--) and GSD VIII rats (—▲—): (A) total glycogen, (B) glycogen isolated from the lysosomal pellet. All values are averaged from three gradients.

TABLE I

Extraction and structure of glycogens isolated from normal and GSD VIII livers

| | *Glycogen (mg/g of liver)* | *Glycogen recovery (%)*[a] | *Protein (mg/g of liver)* | *Protein/ glycogen (%) (w/w)* | *% Glycogen removed by beta-amylase* | *ECL*[b] | *ICL*[c] |
|---|---|---|---|---|---|---|---|
| *Normal* | | | | | | | |
| Liver homogenate | 55.1 | 99 | 155.1 | 286 | – | – | – |
| Purified product | 45.2 | 82 | 1.1 | 2.4 | 57 | 9.8 | 2.2 |
| *GSD VIII* | | | | | | | |
| Liver homogenate | 111.1 | 99 | 150.0 | 135 | – | – | – |
| Purified product | 92.4 | 84 | 1.7 | 1.8 | 59 | 9.8 | 1.6 |

[a] Measured as in ref. 19. [b] External chain length. [c] Internal chain length.

TABLE II

The effect of treatment of glycogen from normal and GSD VIII rats with proteinase K

| | *Glycogen (%)* | | |
|---|---|---|---|
| | *Molecular weight ( × $10^{-6}$)* | | |
| | 0–250 | 250–500 | >500 |
| *Normal* | | | |
| Original | 61 | 16 | 24 |
| + proteinase K | 89 (+46%) | 6 (−63%) | 5 (−80%) |
| *GSD VIII* | | | |
| Original | 88 | 4 | 7 |
| + proteinase K | 93 (+6%) | 4 (0%) | 4 (−43%) |

indicates large differences in the molecular weight distributions of glycogens from normal and GSD VIII animals, and that for the lysosomal glycogen of the latter (Fig. 1B) is biased towards extremely high molecular weight. Table II indicates that high molecular weight, lysosomal glycogen (Fig. 1)[10,25] in GSD VIII animals is more resistant to attack by proteinase K than is the corresponding glycogen from normal animals, which may reflect reduced accessibility of the protein backbone to the enzyme.

*Size and amylolytic degradation.* — From Fig. 2 and Table III, it can be seen that, following cleavage of the disulphide bonds, there was a pronounced increase in the rate

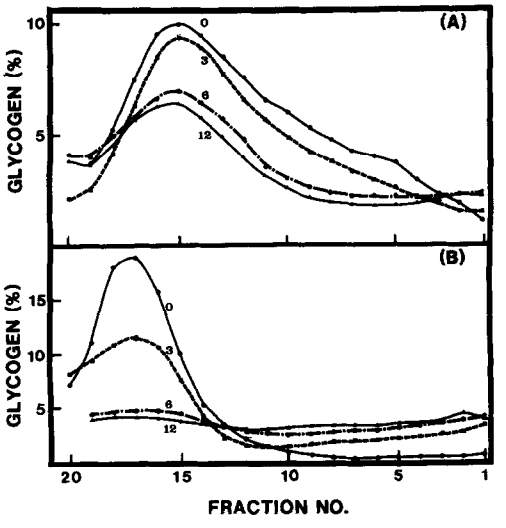


Fig. 2. Time course of size distributions of (A) whole or (B) disulphide-modified glycogens during alpha-amylolysis. Numbers on the curves indicate time (h) of reaction. All values are averaged over three sucrose density-gradients. The results reported in Table III are averages from Fig. 2 and an independent repeat of the experiment.

TABLE III

Time course of treatment of normal and modified glycogen with alpha-amylase

| *Time (h)* | *Glycogen remaining (%)* | | *Relative rate of glucose release (M/N)* | *Glycogen (%)* | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | *Molecular weight ($\times 10^{-6}$)* | | | | | |
| | | | | *0–100* | | *100–400* | | *>400* | |
| | *N*[a] | *M*[b] | | *N* | *M* | *N* | *M* | *N* | *M* |
| 0 | 100 | 100 | 0 | 22.5 | 65.7 | 42.5 | 28.1 | 34.9 | 6.3 |
| 3 | 91.4 | 89.2 | 1.25 | 22.7 | 42.4 | 34.4 | 19.6 | 34.3 | 27.2 |
| 6 | 82.6 | 76.6 | 1.30 | 27.2 | 25.8 | 30.6 | 17.1 | 24.8 | 33.7 |
| 12 | 73.4 | 66.1 | 1.23 | 25.8 | 20.8 | 21.3 | 17.0 | 26.3 | 28.3 |

[a] Normal (pretreatment with 2-mercaptoethanol, then exhaustive dialysis). [b] Modified (treatment with 2-mercaptoethanol, followed by iodoacetamide, then extensive dialysis).

of degradation of the glycogen by alpha-amylase. The data in Table III show a relatively even pattern of alpha-amylolytic degradation of normal, highly polydisperse glycogen, whereas, after pretreatment with 2-mercaptoethanol and iodoacetamide, there was preferential depletion of low molecular weight glycogen. In the latter situation, significant amounts of new high molecular weight material are formed. The data in Table III also show that there is an advantage for alpha-amylolytic degradation in having smaller glycogen molecules, amounting to a 25–30% increase in rate. Attack by alpha-amylase is related to the accessibility of the hydrolysable bonds[39].

However, the results reported here indicate that, since disruption of disulphide bonds does not affect the carbohydrate nature of the individual $\beta$-particles from which glycogen is constructed[13], but merely breaks up the clusters of $\beta$-particles (which are high molecular weight glycogen), the rates of alpha-amylolysis also depend upon the accessible surface area. This view fits well with the concept that the postulated[34] disulphide-bond-breaking capacity of lysosomes permits more rapid degradation of the glycogen. The appearance (Fig. 2) of new high molecular weight glycogen upon alpha-amylolysis of 2-mercaptoethanol/iodoacetamide-modified glycogen suggested that cysteine residues located on the protein backbone[13] were exposed, which allowed new disulphide bonds to be formed. In order to test this hypothesis, glycogen was treated sequentially with 2-mercaptoethanol, iodoacetamide, and alpha-amylase (for 6 h), then re-treated with 2-mercaptoethanol and iodoacetamide. Fig. 3 indicates that the new high molecular weight glycogen is susceptible to attack by disulphide-bond breakers and confirms the formation of new disulphide bonds. This finding supports the concept of improved degradative capacity in lysosomes due to their disulphide-reducing capacity[34].

*Lysosomal glycogen and some metabolites.* — Tables IV and V show the effects of two metabolic stresses (starvation and/or treatment with acarbose) upon liver glycogen and some related metabolites in normal and GSD VIII animals. The persistent effect of a

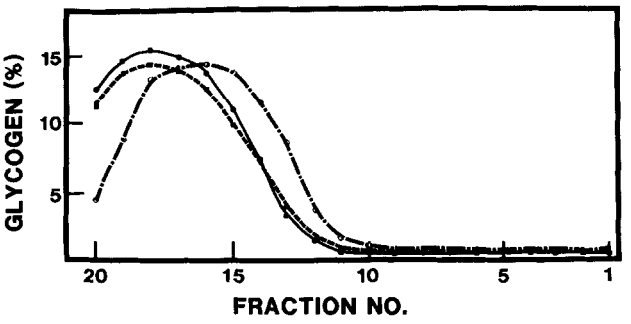


Fig. 3. Size distributions of disulphide-modified, alpha-amylase (6 h)-treated glycogen subsequently re-treated with 2-mercaptoethanol and iodoacetamide. The curves represent the results of three completely independent experiments.

single treatment with acarbose is remarkable, showing increasing glycogen storage over the 5-day period in the lysosomal and cystosolic compartments. Relative changes (Table V) in the total glycogen content of liver appear to correlate in the 1–5-day changes with the levels of blood glucose. In starved and GSD VIII animals, the levels of blood glucose, 5 days after treatment with acarbose, were significantly above normal levels. In contrast, the levels of lactate, and to a lesser extent pyruvate, are depressed. The levels of lactate do not correlate with those of blood glucose. Because lactate is derived from extrahepatic tissue[40], it appears that the increasing levels of blood glucose arise from the disturbance of hepatic glycogen levels by the treatment with acarbose.

Since, over the 5-day period after the treatment with acarbose, the inhibitor concentrates in the lysosomal compartment[22,29–31], immobilising the high molecular weight glycogen within, the feed-back control mechanism controlling the uptake of glycogen must operate[22,31].

In addition, because the GSD VIII animals show $< 10\%$ of normal levels of hepatic phosphorylase[31], these results suggest that, when the catabolism of normal liver glycogen is impaired, blood glucose may be supplied by extra-hepatic tissue, presumably muscle. Further evidence for this suggestion may be adduced from the levels of glucose in tissue, as opposed to those in *blood*, which are, in all cases, less than those observed in the normal control animal and do not appear to bear any direct relation to high levels of blood glucose. This finding again suggests a non-hepatic source (and makes gluconeogenesis, at least in the case of the GSD VIII animals, an unlikely source of the increased levels of blood glucose). Muscle, as the major body store of glycogen, seems the most likely candidate for an extra-hepatic source. Although lacking glucose 6-phosphate[41], muscle stores glycogen in the lysosomal compartment and therefore can liberate glucose, and a relevant modified version of the Cori cycle has been proposed[25]. Muscle tissue is unaffected by treatment with acarbose, in that the inhibitor appears unable to enter muscle cells[29,30]. Therefore, its lysosomal metabolism is unaffected.

The foregoing results emphasise the structure–function relationship between glycogen and its lysosomal metabolism. The structure of glycogen, based on protein

TABLE IV

Effects of treatment with acarbose on tissue glucose, glycogen, and blood metabolites

| | *Time after treatment (days)* | *Total glycogen (mg)* | *Lysosomal glycogen (relative to untreated animals)* | *Tissue glucose (% wet wt. liver)* | *Blood metabolities (mg %)* | | |
|---|---|---|---|---|---|---|---|
| | | | | | *Glucose* | *Pyruvate* | *Lactate* |
| Control | | 385 | 1.00 | 0.27 ±0.02 | 96 ±4 | 0.90 ±0.02 | 12.4 ±1.2 |
| | 1 | 317 | 1.18 | 0.19 | 84 ±2 | 0.28 ±0.04 | 2.8 ±0.5 |
| | 5 | 329 | 1.36 | 0.24 | 104 ±6 | 0.53 ±0.09 | 2.6 ±0.4 |
| Control-starved | | 23 | 1.00 | 0.19 ±0.04 | 63 ±4 | 0.98 ±0.06 | 9.7 ±0.8 |
| | 1 | 33 | 1.02 | 0.20 | 61 ±1 | 0.36 ±0.02 | 5.0 ±0.1 |
| | 5 | 53 | 2.19 | 0.19 | 116 ±3 | n.d.[a] | 8.1 ±0.1 |
| GSD VIII | | 1481 | 1.00 | 0.12 | 86 ±3 | 0.44 ±0.08 | 5.1 ±0.9 |
| | 1 | 1521 | 1.24 | 0.15 | 74 ±4 | 0.56 ±0.03 | 6.7 ±0.3 |
| | 5 | 1677 | 2.74 | 0.14 | 132 ±4 | 0.60 ±0.03 | 7.2 ±0.4 |
| GSD VIII-starved | | 1465 | 1.00 | 0.15 | 76 ±7 | 0.75 ±0.06 | 3.7 ±0.7 |
| | 1 | 1847 | 1.90 | 0.15 | 68 ±2 | 0.56 ±0.03 | 4.5 ±0.4 |
| | 5 | 1946 | 3.93 | 0.12 | 126 ±6 | 0.86 ±0.09 | 4.4 ±0.1 |

[a] Not determined.

TABLE V

Percentage changes in glycogen and metabolites following treatment with acarbose

| | *Time after treatment (days)* | *Total glycogen* | *Lysosomal glycogen* | *Tissue glucose* | *Blood metabolites* | | |
|---|---|---|---|---|---|---|---|
| | | | | | *Glucose* | *Pyruvate* | *Lactate* |
| Control | | | | | | | |
| | 1 | −17 | +18 | −30 | −12 | −69 | −77 |
| | 5 | −14 | +36 | −11 | +8 | −42 | −79 |
| Control–starved | | | | | | | |
| | 1 | +38 | +2 | +5 | −3 | −63 | −48 |
| | 5 | +121 | +119 | 0 | +84 | n.d.[a] | −17 |
| GSD VIII | | | | | | | |
| | 1 | +3 | +24 | +25 | −13 | +27 | +31 |
| | 5 | +13 | +174 | +17 | +55 | +36 | +41 |
| GSD VIII–starved | | | | | | | |
| | 1 | +26 | +90 | 0 | −10 | −25 | +32 |
| | 5 | +33 | +293 | −20 | +15 | +15 | +29 |

[a] Not determined.

backbones with disulphide bonds (a system present in mucins[42]), permits increased rates of degradation of the carbohydrate portion when the disulphide bonds are broken. Increased particle size, as observed in GSD VIII, in contrast, appears to make the protein backbone less accessible. Disturbance of normal lysosomal metabolism by inhibiting glucosidase activity inside the lysosome by the introduction of acarbose upsets the sensitive interactive balance of glycogen levels in the cytosolic and lysosomal compartments in the liver. The resulting imbalance has a flow-on effect observable in metabolites measured in the blood stream. The results re-emphasise the necessity of conducting metabolism experiments on glycogen in any tissue (see, for example, glycogen in yolk sac[43]), in such a way that the separate metabolisms in the cytosol and the lysosome are measured and contrasted.

ACKNOWLEDGMENTS

We thank the Medical Research Council of New Zealand, the Auckland Medical Research Foundation, and the University of Auckland, for financial support, the Ministry of External Relations and Trade for a post-graduate scholarship (to J.P.), and Professor A. G. C. Renwick and Dr G. K. Scott for their continuing support and encouragement.

REFERENCES

1 D. J. Manners, *Adv. Carbohydr. Chem.*, 12 (1957) 261–298.
2 D. J. Manners and A. Wright, *J. Chem. Soc.*, (1962) 1597–1602.
3 O. Kjolberg, D. J. Manners, and A. Wright, *Comp. Biochem. Physiol.*, 8 (1965) 353–365.
4 A. R. Archibald, I. D. Fleming, A. M. Liddle, D. J. Manners, G. A. Mercer, and A. Wright, *J. Chem. Soc.*, (1962) 1183–1190.
5 D. J. Manners, J. J. M. Rowe, and K. L. Rowe, *Carbohydr. Res.*, 8 (1968) 72–81.
6 D. J. Manners, W. H. Schutt, J. R. Stark, and V. Thambyrajah, *Biochem. J.*, 124 (1971) 462–465.
7 S. A. Orrell and E. Bueding, *J. Biol. Chem.*, 239 (1964) 4021–4026.
8 P. Drochmans, *La Morphologie du Glycogene*, Editions Arscia S.A., Brussels, 1966.
9 R. Geddes, *Int. J. Biochem.*, 2 (1971) 657–660.
10 R. Geddes, in G. O. Aspinall (Ed.), *The Polysaccharides*, Vol. 3, Academic Press, New York, 1985, pp. 284–336.
11 D. M. Haverstick and A. H. Gold, *Anal. Biochem.*, 111 (1981) 137–145.
12 T. Iwamasa, T. Tsuru, T. Hamada, and T. Takeuchi, *Acta Histochem. Cytochem.*, 12 (1979) 197–205.
13 R. Geddes, J. D. Harvey, and P. R. Wills, *Eur. J. Biochem.*, 81 (1977) 465–472.
14 R. Geddes and G. C. Stratton, *Carbohydr. Res.*, 57 (1977) 291–299.
15 P. C. Calder and R. Geddes, *Carbohydr. Res.*, 118 (1983) 233–238.
16 P. C. Calder and R. Geddes, *Carbohydr. Res.*, 135 (1985) 249–256.
17 P. C. Calder and R. Geddes, *Carbohydr. Res.*, 148 (1986) 173–177.
18 P. C. Calder and R. Geddes, *Glycoconjugate J.*, 2 (1985) 365–373.
19 P. C. Calder, R. Geddes, and H. Leech, *Glycoconjugate J.*, 3 (1986) 331–338.
20 H. G. Hers, *Biochem. J.*, 86 (1963) 1–8.
21 R. Geddes and G. C. Stratton, *Biochem. J.*, 163 (1977) 193–200.
22 R. Geddes and J. A. Taylor, *Biochem. J.*, 228 (1985) 319–324.
23 P. C. Calder and R. Geddes, *Int. J. Biochem.*, 21 (1989) 569–576.
24 P. C. Calder and R. Geddes, *Carbohydr. Res.*, 191 (1989) 71–78.
25 R. Geddes, *Biosci. Rep.*, 6 (1986) 415–428.
26 D. H. Brown, L. M. Waindle, and B. I. Brown, *J. Biol. Chem.*, 253 (1978) 5005–5011.
27 E. Knecht and J. Hernandez, *Cell Tissue Res.*, 193 (1978) 473–489.

28 G. F. J. M. Vrensen and M. A. C. Kuyper, *J. Microsc. (Paris)*, 8 (1969) 599–614.
29 R. Lullman-Rauch, *Virchows Arch. B*, 38 (1981) 89–100.
30 R. Lullman-Rauch, *Virchows Arch. B*, 39 (1982) 187–202.
31 R. Geddes and J. A. Taylor, *Biosci. Rep.*, 4 (1985) 315–320.
32 S. Ohkuma and B. Poole, *Proc. Natl. Acad. Sci. U.S.A.*, 75 (1978) 3327–3331.
33 J. R. Winkler and H. L. Segal, *J. Biol. Chem.*, 259 (1984) 15369–15372.
34 J. B. Lloyd, *Biochem. J.*, 237 (1986) 271–272.
35 R. S. Malthus and D. G. Clarke, *Proc. Univ. Otago Med. Sch.*, 55 (1977) 31–32.
36 R. S. Malthus, D. G. Clarke, C. Watts, and J. G. T. Sneyd, *Biochem. J.*, 188 (1980) 99–106.
37 J. T. Dingle and A. J. Barrett, in J. T. Dingle and H. B. Fell (Eds.), *Lysosomes in Biology and Pathology*, Vol. 2, North-Holland, Amsterdam, 1969, pp. 555–556.
38 M. Bradford, *Anal. Biochem.*, 72 (1976) 248–254.
39 R. Geddes, *Carbohydr. Res.*, 7 (1968) 493–497.
40 C. F. Cori, *Biol. Symp.*, 5 (1941) 131–140.
41 C. T. Cori, and C. F. Cori, *J. Biol. Chem.*, 131 (1939) 297–298.
42 R. Shogren, A. M. Jamieson, J. Blackwell, P. W. Cheng, D. G. Dearborn, and T. F. Boat, *Biopolymers*, 22 (1983) 1657–1675.
43 P. J. Hartfield, K. E. Williams, R. Geddes, and J. B. Lloyd, *Placenta*, 10 (1989) 45–54.