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COMPARTMENTATION OF GLYCOGEN METABOLISM IN THE LIVER

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ABSTRACT

The incorporation of radioactivity into liver glycogen has been shown not only to be a metabolically inhomogeneous process but also to depend critically on the nature of the precursor. D-Galactose is incorporated into glycogen by a mechanism which is separate from that associated with the incorporation of D-glucose. D-Galactose is favoured for incorporation into high-molecular-weight glycogen and consequently is affected more by treatment of the animal with the antibiotic tunicamycin, since high-molecular-weight glycogen is preferentially found in the lysosomal compartment.

INTRODUCTION

Recently, it has been shown that the incorporation of 2-amino-2-deoxy-D-galactose (D-galactosamine) into liver glycogen as its glucose analogue is a metabolically inhomogeneous process after starvation[1]. It has also been reported that D-galactosamine is metabolised in liver by the same pathway as D-galactose[2–4]. Therefore, it seemed of interest to compare the incorporation of D-galactose and D-glucose. The resynthesis utilising D-galactosamine appears to be associated with the ribosomes or the rough endoplasmic reticulum[4] as does that with D-glucose under similar circumstances[5].

Glycogen is well established as being metabolically and structurally inhomogeneous in the liver[1,6–17] and other tissues[18,19]. It is found in the form of single spheres (β-particles[20]) or covalently constructed aggregates of these spheres (α-particles[20]). Although the carbohydrate construction of the spheres within the α-particles is well elucidated[21] and fairly uniform[22], the covalent linkage to the protein backbone[23] appears to be limited to the high-molecular-weight material[1]. This latter material is also associated with the lysosomal compartment of the cell[12,13] and binds glycogen synthase in preference to phosphorylase[24].

EXPERIMENTAL

New Zealand white rats (Wistar type) were used. Livers were removed rapidly, after cervical dislocation, and plunged into liquid nitrogen in order to

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eliminate the effects of the rapid, metabolically inhomogeneous, post-mortem degradation[14]. Glycogen was isolated by a cold-water extraction method and fractionated on sucrose density-gradients as previously described[12]. Diffusion coefficients had previously been determined for glycogen fractions by laser intensity-fluctuation spectroscopy[15,16], and molecular weights were obtained by application of the Svedberg equation[15].

Concentrations of glycogen solutions were determined by a calcium chloride-enhanced iodine–iodide reaction[12] and those of protein by a modification of the Lowry method[25].

Lysosomal glycogen was isolated by the technique of Geddes and Stratton[12].

RESULTS AND DISCUSSION

As can be seen from Fig. 1, there was a radically different distribution of radioactivity in normally fed rats when D-glucose or D-galactose was provided as a glycogen precursor, regardless of whether the isotope was introduced intraperitoneally or intravenously. Radioactivity originating from galactose had a much greater tendency to be found in high-molecular-weight glycogen. Since it is well established that a significant proportion of normal hepatic glycogen metabolism

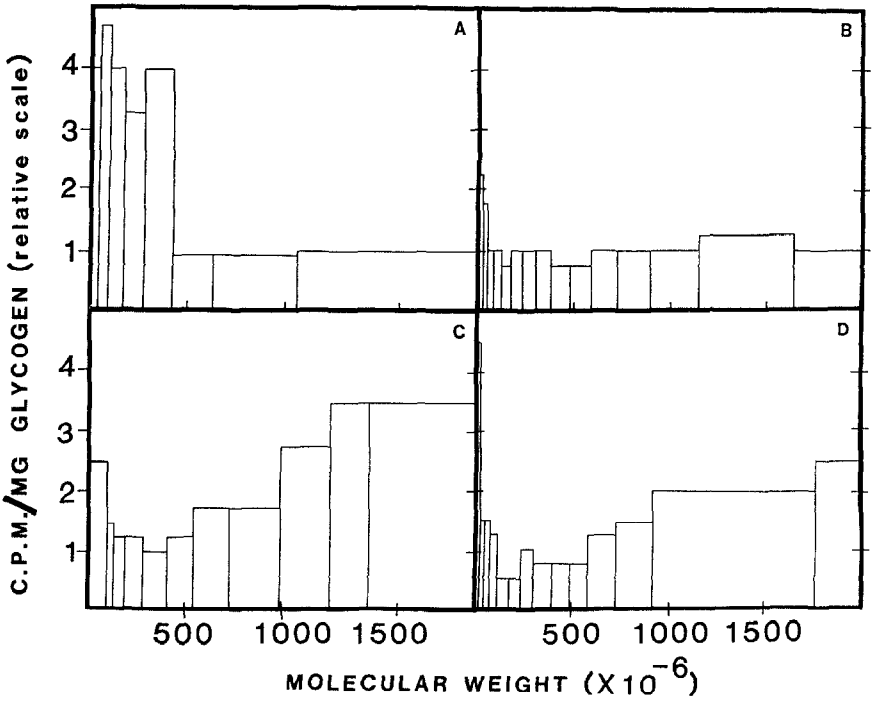

Fig. 1. Radioactive incorporation into liver glycogen with respect to molecular weight. Normally-fed Wistar rats were injected with either D-[U-^{14}C]glucose (A,B) or D-[U-^{14}C]galactose (C,D), intraperitoneally (A,C) or intravenously (B,D), and sacrificed after 3 h.

proceeds *via* the lysosomal compartment[12,13], glycogen was also isolated from a lysosome-enriched sub-cellular fraction[12]. The results (Fig. 2) again show clearly the preferential incorporation of D-galactose into high-molecular-weight glycogen, in contrast to the incorporation of D-glucose. This finding confirms the metabolic heterogeneity of glycogen with respect to molecular size[8,10,12].

Devos and Hers[17,26–28] have proposed a molecular order in hepatic synthesis and degradation. Since these authors utilised mostly D-galactose as the precursor and took no account of metabolic heterogeneity, their experimental procedures[17] were repeated and the results examined in relation to molecular size of glycogen (Fig. 3). After the starvation/refeeding regime, early incorporation was mainly into low-molecular-weight glycogen with the effect being much more marked and intense with galactose. These results must be considered in conjunction with the data in Table I where the marked heterogeneity of the resynthesis in regard to molecular weight and amount is shown. During starvation, the glycogen stores are heavily depleted and the glycogen is greatly reduced in molecular size[8,10]. As observed before[1,10], the intense, post-starvation resynthesis of the glycogen stores

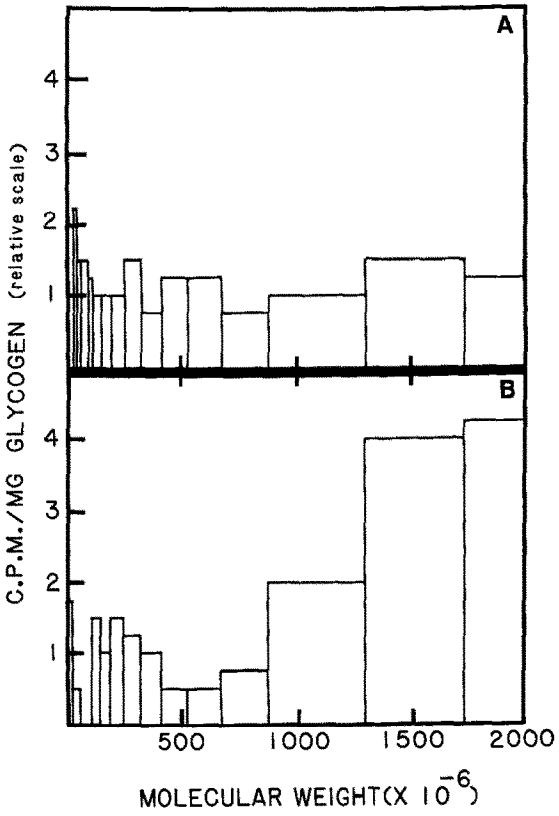

Fig. 2. Distribution of radioactivity incorporated into lysosomal glycogen utilising D-[U-^{14}C]glucose (A) or D-[U-^{14}C]galactose (B) as precursor.

favours initially the synthesis of high-molecular-weight material (after 3 h, there was a 27-fold increase in glycogen of molecular weight $>500 \times 10^6$ as compared to a 5–6 fold increase in the low-molecular-weight range), but continued resynthesis subsequently overproduced low-molecular-weight glycogen[1,10]. The resynthesis was accompanied by a ~50% increase in the protein–polysaccharide ratio[1,9]. Fig. 3 indicates that the incorporation of radioactivity from glucose and galactose proceeds by somewhat different routes. In the intense metabolic activity following refeeding, radioactivity from glucose was lost over all the glycogen-size-range, whereas the high-molecular-weight material synthesised with radioactivity from a galactose precursor was retained even after 9 h. The loss of the glucose-originating activity reflects their depletion during the normal functioning, in the cytosol, of a glycogen synthase/phosphorylase futile cycle, as has been reported[29,30]. The "stable" radioactivity in high-molecular-weight glycogen arising from the galactose precursor is not involved in this cycle, *i.e.*, it is in a separate compartment, presumably the lysosome[12,13].

Further evidence of the complexity of the involvement of glycogen metabolism with the lysosomal compartment was provided by the results of the treatment of the animal with tunicamycin. This nucleoside antibiotic produced by *Streptomyces lysosuperificus*[31] not only blocks the first step in the pathway of the synthesis of the oligosaccharide portion of asparagine-linked glycoproteins[32], but

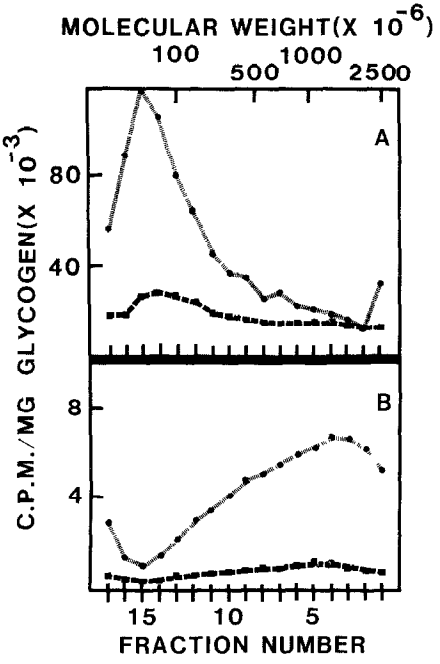

Fig. 3. Incorporation of radioactivity into liver glycogen, after a period of starvation followed by refeeding[17]. The radioactive precursor was either D-[U-^{14}C]glucose (---■---) or D-[U-^{14}C]galactose (IIIII●IIIII). The periods of refeeding were 0 5 (A) or 9 h (B).

TABLE I

HETEROGENEITY OF GLYCOGEN RESYNTHESIS AFTER A PERIOD OF STARVATION AND VARIED PERIODS OF REFEEDING, USING THE CONDITIONS DESCRIBED BY DEVOS AND HERS[17]

Period of refeeding (h)		0.5	3	9
Glycogen (%)	mol. wt. <500	86	55	76
	mol. wt. >500	14	45	24
Relative[a] amount	mol. wt. <500	6	33	111
	mol. wt. >500	1	27	35

[a]As g/g of wet liver; mol. wt. >500 after 0.5-h refeeding, taken as 1.

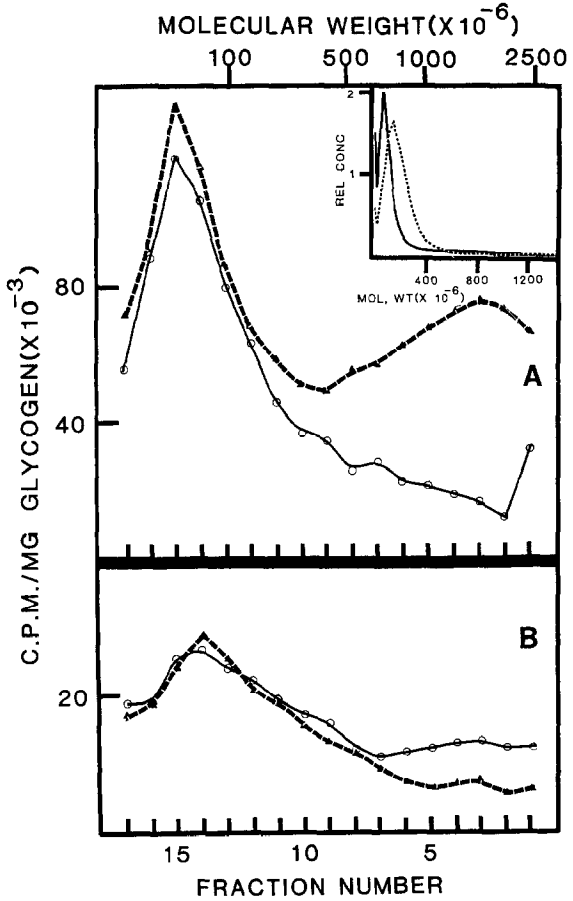

Fig. 4. The effect of tunicamycin on the incorporation of radioactivity into glycogen after refeeding 0.5 h after starvation (conditions as for Fig. 3). The radioactive precursor was either D-[U-^{14}C]galactose (A) of D-[U-^{14}C]glucose (B); tunicamycin-treated, ---▲---; control, ———○———. The insert shows the molecular weight distribution[15] of tunicamycin-treated (------) and control (———) glycogens.

also interferes with the secretory processes associated with the endoplasmic reticulum-Golgi-lysosome compartments[33]. Since it would seem unlikely, by the nature of the synthesis involved[34], that glycogen is linked to protein[9,11,23] through nitrogen, then tunicamycin should only affect glycogen secretion. As shown in Fig. 4, not only is the overall size distribution grossly affected but again there are large differences in the incorporation of galactose and glucose into glycogen. Tunicamycin, by affecting secretion, would be expected to affect the overall glycogen-molecular-weight distribution (Fig. 4b) since interference with lysosomal glycogen metabolism, whether by targetting of antibodies[13] or by the use of acarbose[35], has been shown to have a feedback effect on glycogen synthesis.

All the results presented above indicated that there was clearly a different route of incorporation of radioactivity from galactose into glycogen as compared to the glucose route, and points to a pronounced compartmentalisation of glycogen metabolism. Radioactive D-galactose, after being presented to the cell, is converted into the 1-phosphate and thence into the nucleotide sugar by exchange with UDP-D-glucose[2–4]. Subsequent epimerisation of the radioactive molecule provides radioactive UDP-D-Glc as a substrate for glycogen synthase[36]. Incorporation of radioactivity from D-glucose passes through the 6- and 1-phosphates. Our experiments showed these two routes to incorporation into glycogen to be independent of one another, *i.e.*, in separate compartments. Glycogen is already known to be heterogeneous with respect to (*a*) molecular weight[6,8,15], (*b*) construction (α- and β-particles)[9–11], (*c*) molecular structure (protein content)[1–9], and (*d*) cellular location[12–14]. These results show that heterogeneity in synthesis must also be taken into account when assessing glycogen metabolism. The molecular order in the synthesis and degradation of glycogen in the liver observed by Devos and Hers[17,26–28] is clearly not a molecular effect, as suggested, but a consequence of the compartmentalisation of glycogen synthesis and degradation described above. The results of electron microscopy published later in support of the molecular hypothesis[28] may be discounted, since ~50% of the glycogen was lost during the fixation process[37] and this post-mortem loss is known to deplete high-molecular-weight glycogen preferentially[14].

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REFERENCES

1 P. C. CALDER AND R. GEDDES, *Carbohydr. Res.*, 118 (1983) 233–238.
2 F. MALEY, J. F. MCGARRAHAN, AND R. DELGIACCO, *Biochem. Biophys. Res. Commun.*, 23 (1966) 85–91.

3 F. MALEY, A. L. TARENTINO, J. F. MCGARRAHAN, AND R. DELGIACCO, *Biochem. J.*, 107 (1968) 637–644.
4 A. L TARENTINO AND F. MALEY, *FEBS Lett.*, 71 (1976) 215–219.
5 G. F. J. M. VRENSEN, *J. Microsc. (Paris)*, 9 (1970) 517–534.
6 M. R. STETTEN AND D. STETTEN, *J. Biol. Chem.*, 207 (1954) 331–340.
7 M. R. STETTEN AND D. STETTEN, *J. Biol. Chem.*, 213 (1955) 723–732.
8 R. GEDDES, *Int. J. Biochem.*, 2 (1971) 657–660.
9 N. P. CHEE AND R. GEDDES, *FEBS Lett.*, 73 (1977) 164–166.
10 R. GEDDES AND G. C. STRATTON, *Carbohydr. Res.*, 57 (1977) 291–299.
11 R. GEDDES, J. D. HARVEY, AND P. R. WILLS, *Eur. J. Biochem.*, 81 (1977) 465–472.
12 R. GEDDES AND G. C. STRATTON, *Biochem. J.*, 163 (1977) 193–200.
13 R. GEDDES, D. E. OTTER, G. K. SCOTT, AND J. A. TAYLOR, *Biochem. J.*, 212 (1983) 99–103.
14 R. GEDDES AND K. B. RAPSON, *FEBS Lett.*, 31 (1973) 324–326.
15 R. GEDDES, J. D. HARVEY, AND P. R. WILLS, *Biochem. J.*, 163 (1977) 201–209.
16 J. D. HARVEY, R. GEDDES, AND P. R. WILLS, *Biopolymers*, 18 (1979) 2249–2260.
17 P. DEVOS AND H.-G. HERS, *Biochem. J.*, 192 (1980) 177–181.
18 N. P. CHEE, R. GEDDES, AND P. R. WILLS, *Biochim. Biophys. Acta*, 756 (1983) 9–12.
19 P. C. CALDER AND R. GEDDES, *Carbohydr. Res.*, 135 (1985) 249–256.
20 P. DROCHMANS, *J. Ultrastruct. Res.*, 6 (1962) 141–163.
21 H. M. BULLIVANT, R. GEDDES, AND P. R. WILLS, *Biochem. Int.*, 6 (1983) 497–506.
22 J.-C. WANSON AND P. DROCHMANS, *J. Cell. Biol.*, 38 (1968) 130–150.
23 C. R. KRISMAN AND R. BARENGO, *Eur. J. Biochem.*, 52 (1975) 117–123.
24 A. A. BARBER, S. A. ORRELL, AND E. BUEDING, *J. Biol. Chem.*, 247 (1967) 4040–4044.
25 O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR, AND R. J. RANDALL, *J. Biol. Chem.*, 193 (1951) 265–275.
26 P. DEVOS AND H.-G. HERS, *Eur. J. Biochem.*, 99 (1979) 161–167.
27 P. DEVOS AND H.-G. HERS, *Biochem. Biophys. Res. Commun.*, 95 (1980) 1030–1036.
28 P. DEVOS AND H.-G. HERS, *Biochem. J.*, 209 (1983) 159–165.
29 J. D. NEWMAN AND J. MCD. ARMSTRONG, *Biochim. Biophys. Acta*, 544 (1978) 225–233.
30 H.-G. HERS, *Biochem. Soc. Trans.*, 4 (1976) 985–988.
31 A. TAKATSUKI, K. ARIMA, AND G. TAMURA, *J. Antibiot.*, 24 (1971) 215–223.
32 J. TKACZ AND J. O. LAMPEN, *Biochem. Biophys. Res. Commun.*, 65 (1975) 248–257.
33 S. HICKNAN, A. KULCZSKI, R. G. LYNCH, AND S. KORNFELD, *J. Biol. Chem.*, 252 (1977) 4402–4409.
34 R. T. SCHWARZ AND R. DATEMA, in M. I. HOROWITZ (Ed.), *The Glycoconjugates*, Vol. 3, Academic Press, New York, 1982, pp. 47–79.
35 R. GEDDES AND J. A. TAYLOR, manuscript submitted *Biochem. J.*
36 P. COHEN, *Nature (London)*, 296 (1982) 613–620.
37 R. GEDDES AND H. LEECH, unpublished results.