NUCLEAR GLYCOGEN SYNTHASE – AN ARTIFACT OF PREPARATION

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1. Introduction

Nuclear deposits of glycogen, are usually related to pathological conditions. These include human hepatocytes following prednisolone treatment [1], in liver biopsies obtained in cases of hepatitis [2,3], in Novikoff hepatoma cells [4] and in Erlich ascites [5–8] where glycogen synthase was reported in purified nuclei [9] and the accumulation of nuclear glycogen demonstrated by electron microscopy and autoradiography [10]. In a limited number of reports an abnormal deposition of glycogen in the nucleus was linked with diabetes [11]. We have examined the activity of glycogen synthase in liver nuclei of normal and diabetic rats. Here we demonstrate low levels of nuclei-associated activity of glycogen synthase. The electron-microscopic data indicate that glycogen synthase activity associated with liver nuclei is most probably an artifact of preparation.

2. Materials and methods

Male Wistar rats (250–350 g) fed ad libitum, were used throughout. Livers perfused free of blood were rapidly homogenized and nuclei isolated as in [12]. Briefly, nuclei were sedimented by ultracentrifugation from 10% homogenates prepared in 2.2 M sucrose, 3 mM $CaCl_2$ and 0.5 mM Tris–HCl (pH 7.4). The nuclear pellet was resuspended in 1 M sucrose, 1 mM $CaCl_2$, 0.5 mM Tris–HCl (pH 7.4) sedimented by centrifugation (3000 × *g* for 5 min) and resuspended in the same medium. Whole homogenates were assayed after dilution (with 0.5 mM Tris–HCl (pH 7.4) 2.2-fold. Glycogen synthase was assayed as in [13] at higher specific radioactivity of UDP-[^{14}C] glucose. Assays were incubated at 30°C in triplicate. Mild diabetes was induced by treating rats with streptozotocin (50 mg/kg body wt) and monitored by urine volumes. The treated animals were able to maintain themselves showing little, or no weight gain when compared to control animals. Nuclear pellets were fixed and processed for electron microscopy as in [14]. The sources of chemicals and radiochemicals were as in [14,15]. All other chemicals were of analytical grade.

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3. Results

The specific activity of glycogen synthase (I + D form, assayed in the presence of 6.6 mM glucose 6-phosphate) in frozen and thawed nuclear fractions was ~10–15% of that of the homogenate (table 1). The total activities, however, were only 0.3–0.4% of the total cellular activities of the enzyme. Both the specific activities and the ratio of nuclear to total glycogen synthase did not change significantly in diabetic rats and diabetic rats treated with glucose and insulin (5 U/Kg body wt) (14 days following treatment with streptozotocin).

Nuclear glycogen synthase was assayed immediately following the isolation procedure (~4 h after sacrifice) and the homogenate glycogen synthase was assayed immediately following homogenization (20 min after sacrifice) and again together with the nuclear fraction. The independent activities of glycogen synthase increased 2–3-fold in the homogenate during the 3.5 h required for subsequent isolation of the nuclear fraction. Nevertheless, nuclei suspended in the same medium for the same time and the same temperature (0–4°C) consistently displayed a lower independent activity of glycogen synthase than the enzyme in

Table 1
Fraction and time of assay

	Homogenate		Nuclei
	∿20 min	∿4 h	∿4 h
Normal rats			
Spec. act.			
(nmol . mg^{-1} . min^{-1})	2.97 ± 1.61 (18)	4.01 ± 2.56 (14)	0.59 ± 0.58 (9)
% I	18.1 ± 4.9 (14)	50.4 ± 10.5 (14)	11.9 ± 5.5
% of total	–	–	0.36 ± 0.14
Diabetic			
Spec. act.			
(nmol . mg^{-1} . min^{-1})	3.9 (2)	4.6 (2)	0.45 (2)
% I	11 (2)	32 (2)	7.5 (2)
% of total	–	–	0.27 (2)
Diabetic insulin-treated			
Spec. act.			
(nmol . mg^{-1} . min^{-1})	3.3 (2)	4.1 (2)	0.51 (2)
% I	15 (2)	37 (2)	14 (2)
% of total	–	–	0.41 (2)

Purified nuclear fraction and liver homogenates were assayed at the indicated times (following sacrifice) as in section 2. Insulin-treated rats were injected with 3 g/kg glucose i.p. and 5 u/kg insulin i.v. and sacrificed 2.5 h later. The results are the near of a number of independent experiments (in parentheses ± SEM

homogenates assayed immediately after homogenization (table 1). This indicates that the enzyme associated with nuclei is protected from the glycogen synthase phosphoprotein phosphatase.

Electron microscopic examination of the nuclear fraction revealed a preparation which contained mostly intact nuclei and very little contaminating material. Glycogen particles were detected in many nuclei. However, the glycogen particles were consistently associated with nuclei which displayed damage to the nuclear envelope (fig.1a–d). Moreover, the same nuclei which contained glycogen particles also contained other cellular debris (e.g., smooth membranes and rough endoplasmic reticulum). No glycogen was detected in nuclei in fixed liver sections [16]. It is apparent that damage to the nuclear envelope results in a non-specific trapping of cellular contents in the partially ruptured nuclei.

4. Discussion

The accumulation of glycogen in cell nucleus has been ascribed to the transfer of cytoplasmic glycogen to the nucleus. The demonstration of glycogen synthesis in Erlich ascites nuclei [9,10] and massive deposits of glycogen in liver nuclei in biopsies obtained from diabetics suggested the possibility that nuclear synthesis of glycogen might be a feature in some cells. In other cells the enzymes involved in synthesis might be latent and triggered only by a pathological condition (e.g., hepatitis or diabetes).

Our observations point to the following conclusions:

1. The activities of glycogen synthase associated with a purified nuclear preparation are below the level of contamination of the preparation by cytoplasmic content* (0.5–1%, according to [12].
2. Although glycogen particles can be detected inside isolated liver nuclei, they are consistently present in damaged nuclei and accompanied by the presence of other cytoplasmic structures.
3. Glycogen synthase associated with the nuclear

* An incomplete perfusion of the tissue to free it from most of the blood might be a possible source of error. The nuclear pellets from unperfused and partly-perfused livers were extensively contaminated with glycogen and, consequently, very high activity of glycogen synthase

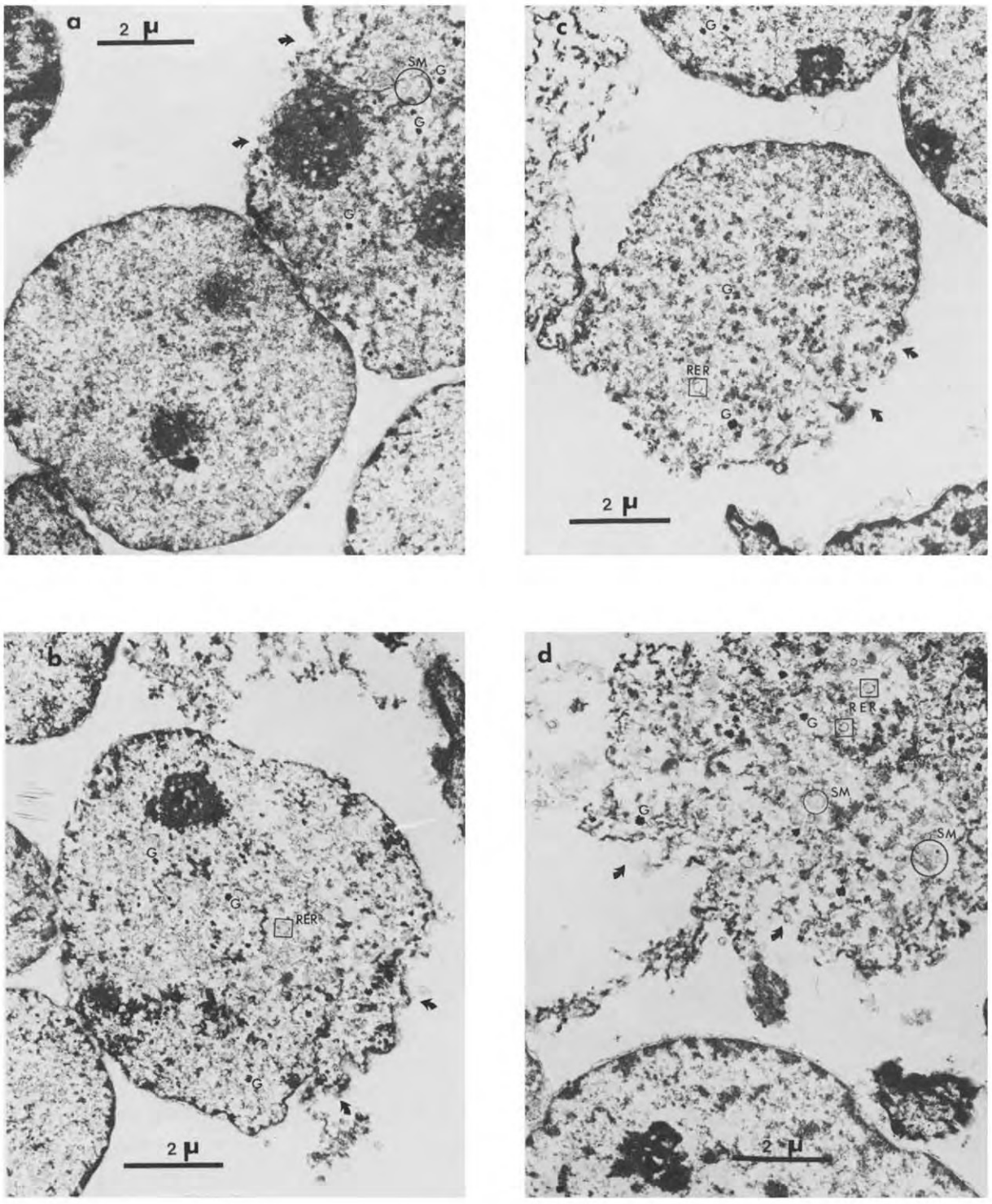

Fig.1. (a–d). Micrograph of purified liver nuclei prepared as in section 2. Black arrows indicate nuclei with visible damage of nuclear envelope: Glycogen particles (G); rough endoplasmic reticulum (RER); smooth membranes (SM).

fraction appears to be protected from the action of cellular glycogen synthase phosphatase.

We conclude that the low activity detected in the nuclear fraction is most probably due to glycogen synthase adsorbed on glycogen particles trapped with other cytoplasmic structures in damaged nuclei. The trapping will explain the resistance of the enzyme to the action of cytoplasmic phosphatase. The presence of glycogen particles in nuclei and/or the high activity of glycogen synthase associated with purified nuclear fraction might indicate a preparation containing a high proportion of damaged nuclei and might be a useful criterion for the degree of the damage.

There was no significant change in the activity of glycogen synthase in nuclear preparations from untreated and insulin-treated streptozotocin-diabetic rats. This, however, does not exclude the possibility of glycogen accumulation in the nuclei of tissues of diabetics suffering from this disorder for an extended period of time.

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