POST-MORTEM DEGRADATION OF GLYCOGEN

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1. Introduction

Although glycogen is the most extensively studied of the polysaccharides of animal tissue there has been no previous investigation of the effect of post-mortem changes on the distribution of molecular sizes of glycogen in tissue. Available data has described the changes in the total glycogen content [1–3]. Further, two recent papers [4, 5], examined the structure of a glycogen isolated from human autopsy material "within 2 hr of death" ([5], p. 1366). It was therefore of great importance for the continuing study of "native" glycogen [6], to investigate the changes which occur in tissue glycogen after death both with respect to total amount and also with respect to size distribution.

2. Experimental

The animals used were New Zealand white rats and the study was confined to liver since this is the most abundant source of glycogen and also the most investigated tissue. The livers were removed from the control rats within 30 sec of cervical dislocation and immediately placed in liquid nitrogen. Livers from other rats were allowed to remain in the dead animal for periods of up to 2 hr before removal and placement in liquid nitrogen. Small portions of liver were removed simultaneously and the glycogen and glucose contents measured by the method of Kemp and van Heijningen [7].

The frozen liver was homogenised in 45% phenol for 4 min then stirred gently over 1 hr [8, 9]. The phenol solution was then allowed to layer and the upper aqueous layer containing glycogen and RNA was removed. After dialysis the RNA was removed by treatment with ribonuclease overnight at room temp. Full details of this method will be published elsewhere [10].

Purified and concentrated glycogen solutions were placed on essentially linear sucrose density gradients [11] and centrifuged in a swinging bucket rotor (Beckman Spinco SW 25.2) (Gradient 50 ml, sample 3 ml, layered on top) at 15,000 rpm for 30 min. Fractions of approx. 2.4 ml were removed from the bottom of the tubes using the device of Tan [12] and analysed for glycogen using the iodine–iodide reaction [10, 13].

3. Results

Fig. 1 indicates that there is an initial rapid decrease in the total glycogen content followed by a slower but still very significant decrease. The rapidity of the initial decrease indicates that the utmost speed must be used in inhibiting the enzymic reactions which proceed upon death of the animal, if any relation to the glycogen content of the normal animal is to be expected. The increase in the free glucose shown will be paralleled by other products of glycolysis.

Fig. 2 clearly indicates that the degradation of the glycogen is a metabolically inhomogeneous process in that all the molecular sizes of glycogen are not degraded at the same rate. Metabolic inhomogeneity of glycogen synthesis has already been shown [6]. The calculation [14] of the sedimentation coefficients shown on the upper border of the figure uses a value of 0.68 for the partial specific volume. (Derived from

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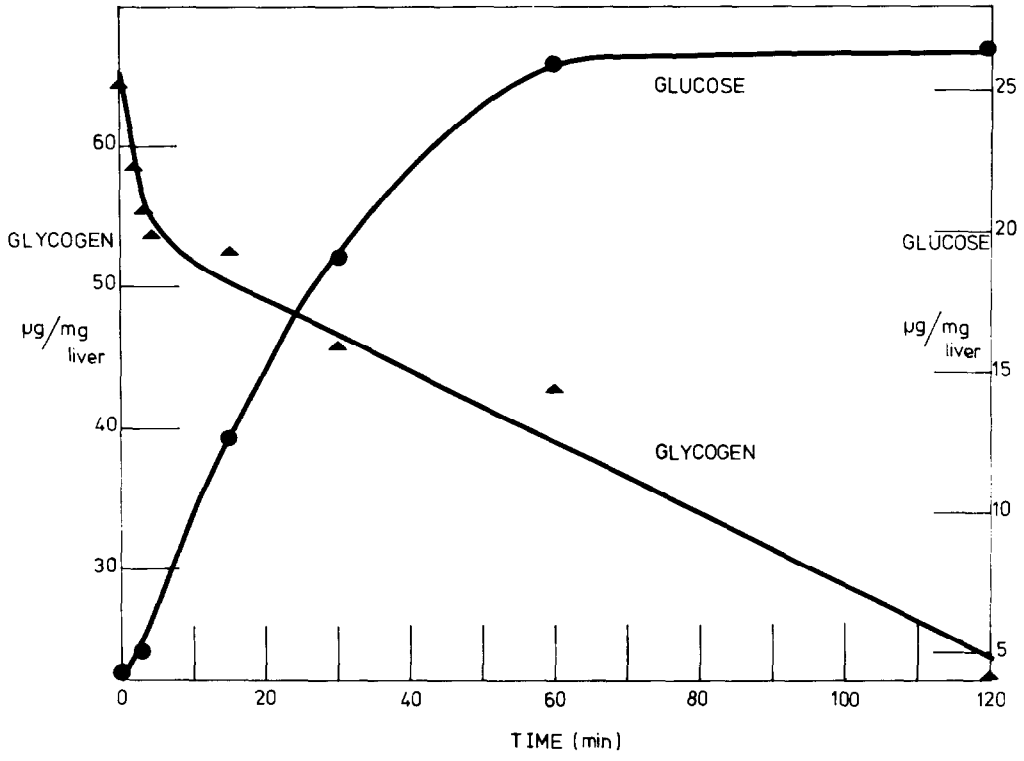

Fig. 1. Changes in total liver content of glycogen and free glucose at various times after death. Each point is averaged from at least three livers.

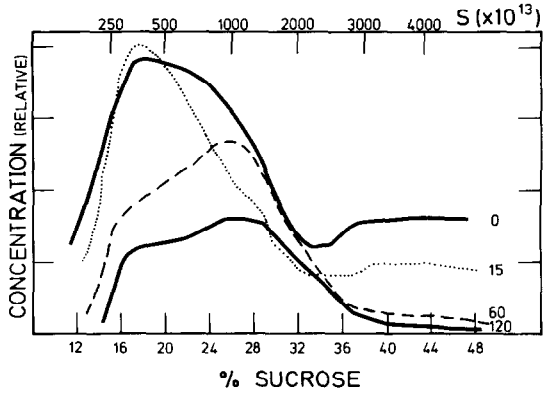

Fig. 2. Effect of post-mortem degradation on the size distribution of liver glycogen. The times (in min) after death are indicated beside the respective curves. The zero time curve is averaged from 4 livers. All other curves averaged from 2 livers. Approximate sedimentation coefficients are indicated along the top of the figure.

equilibrium density gradient bonding in sodium iothalamate [10, 15]).

If 2000 S is considered arbitrarily to be a division between "low" and "high" molecular weight glycogen then, from table 1 it can be seen that during the initial rapid post-mortem degradation high molecular weight glycogen is preferentially degraded. This loss of the large glycogen molecules is continued at later times but is paralleled by loss of its smaller sized counterparts. It should be noted that practically all of the material above 3500 S is absent after the first hour.

The reasons for the non-homogeneous breakdown of glycogen are probably related to the molecular structure of glycogen which, from electron-microscopy [16, 17], has been shown to form both single spherical particles (β-particles; diameters 20–60 nm) and also large clusters of these spherical particles (α-particles). From the theoretical calculations of Pollard [18] and Stetten [19] who indicated that the *maximum* possible molecular weight of a simple

Table 1
Post-mortem degradation of glycogen.

Time elapsed before extraction (min)	Original glycogen content remaining (%)	Original glycogen content from 0–2000 S remaining (%)*	Original glycogen content > 2000 S remaining (%)*	Glycogen > 2000 S (%)*
0	100	100	100	27
15	77	83	61	22
60	60	63	31	16
120	38	39	22	18

* Calculated from the areas beneath the curves in fig. 1 (planimeter).

glycogen molecule, i.e. a β-particle, would be about 140×10^6, it is clear that the peaks in fig. 2 below 2000 S must be largely composed of a range of sizes of β-particles. (A value of 1000 S when substituted in the Svedberg equation would give a molecular weight of the order of 100×10^6 – using a diffusion coefficient of ca. 0.7×10^{-7} calculated from laser light-scattering experiments [20]). The difference in affinity of some enzymes for large and small molecular weight glycogen has already been shown [21] and the preferential degradation of α-particles seems likely to be a related phenomenon.

In conclusion it has been clearly shown that speed of removal of the tissue after death and immediate inhibition of the enzyme systems (by freezing, in this case, at liquid nitrogen temperatures) is essential if glycogen related to that found *in vivo* is to be extracted. It appears that degradation is somewhat related to the architecture of the glycogen particle, an observation which is presumably relevant in the normal process of glycolysis in the live animal. The authors cautiously suggest that the application of the above techniques might be of significance in forensic medicine.

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