Relation Between the Glycogen Content of the Liver and Liver Weight, and its Meaning for Enzymology

Rat liver weight is regarded to be age-dependent[1,2]. In this paper we show that liver weight largely depends on the glycogen content of the liver. This finding has consequence for the estimation of enzyme activity in the liver. The mode of expressing enzyme activity has drawn a lot of attention[3–7]. It is generally agreed that enzyme activity in tissues has to be expressed per mg of protein. However, quite often enzyme activity is still expressed per gram liver weight. In this paper we show that significant changes in enzyme activity can be obtained if enzyme activity is expressed per gram wet liver tissue while the activity of the whole liver has not been changed.

Materials and methods. Male white rats (Wistar WU) weighing 275–325 g were fed with a carbohydrate-rich diet (bread) for 6 days. Thereafter the animals were starved. After weighing the animals, livers were excised under ether narcosis and weighed. Glycogen was estimated according to the phenol sulphuric acid method[8]. Water was measured gravimetrically by drying at 120 °C. Fatty acids were determined titrimetrically after saponification[9]. The protein content was determined by the Biuret method, correcting for turbidity due to fat or glycogen according to the method of HENRY[10].

One fragment of liver tissue was fixed in Carnoy's liquid and celloidin sections were prepared and stained with carmine (Chroma Gesellschaft, Germany). The mean cell diameter was estimated with an ocular micrometer by measuring the largest diameter of 2×10 cells in two different central areas in 6 animals.

Glycogen synthetase (EC 2.4.1.11) activity. The enzyme activity was estimated according to LELOIR and GOLDEMBERG[11].

Phosphorylase (EC 2.4.1.1.) activity. Frozen liver tissue was homogenized in 0.1 *M* NaF (1 g liver tissue in about 9 ml 0.1 *M* NaF, pH 6.7) at 0 °C in a VirTis '45'. A sample was incubated in 1% glycogen, 0.1 *M* NaF (pH 6.7) and 0.015 *M* G-1-P (final concentrations). The reaction was stopped by addition of TCA (final concentration 11.5%). Inorganic phosphate in the supernatant was measured according to TAUSSKY and SHORR[12]. The activity of phosphorylase a+b was estimated by addition of 0.005 *M* ATP-$MgCl_2$ to the incubation medium.

Hexokinase (EC 2.7.1.1) and glucokinase (EC 2.7.1.2) activity. The activity of these enzymes was estimated according to a modification of the method of VIÑUELA et al.[13]. Modifications of the method: a) livers were not perfused and b) activity was estimated in a homogenate instead of in an 'extract'.

Glucose-6-phosphatase (EC 3.1.3.9) activity. Liver tissue was homogenized in 0.1 *M* NaF (pH 6.5) at 0 °C in a VirTis '45' homogenizer for 5 min. A sample was incubated in a Labline water bath for 10 min. The incubation medium contained 0.05 *M* G-6-P and 0.1 *M* citrate buffer. The reaction was stopped with TCA (final concentration 11.5%). Phosphate in the supernatant was estimated according to TAUSSKY and SHORR[12].

Results and discussion. During a carbohydrate-rich diet the weight of the liver comprises 4–5% of the body weight

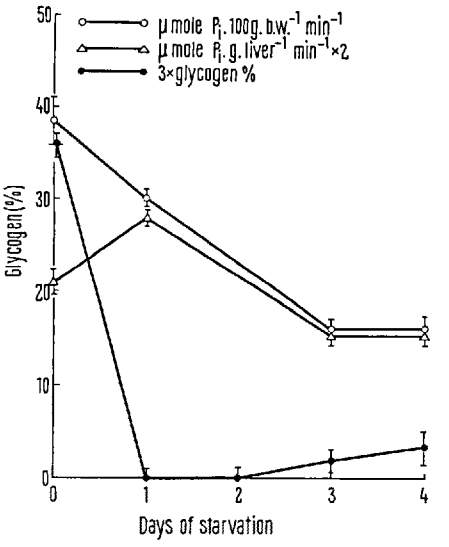

Fig. 2. Glycogen percentage and phosphorylase activity on the basis of liver weight and body weight vs. the starvation time. Glycogen has disappeared whereas the activity of phosphorylase a/g liver has increased after 24 h of starvation. However, if enzyme activity is expressed per 100 g body wt., phosphorylase activities, as well as the glycogen percentage of the liver, have decreased after 24 h of starvation. Each result is the mean of 6 animals.

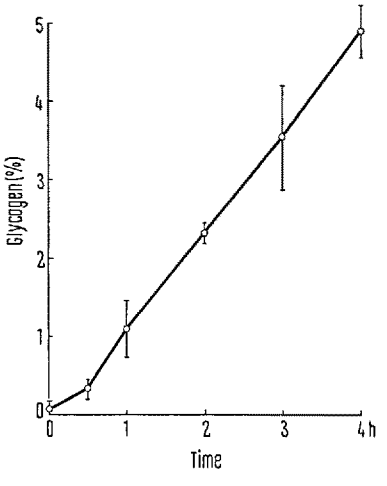

Fig. 1. Deposition of liver glycogen in rats fed with a carbohydrate-rich diet after 24 h of starvation. Each result is the mean of 6 animals.

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Table I. Water, glycogen, fatty acid and protein content of the liver after a carbohydrate-rich diet during 6 days and the same diet followed by 24 h of starvation. Each result is the mean of 6 animals.

	Carbohydrate-rich diet %	+24 h of starvation %
Water	69	69
Glycogen	14	0
Fatty acid	2	2
Protein	14	24

Table II. Liver weight and cell diameter in glycogen-rich livers and in livers without glycogen

	A Carbohydrate-rich diet	B +24 h of starvation	A/B
Glycogen (%)	16.1	0	
Weight of 6 livers (g)	89.72	42.30	2.1
Cell diameter	17.56	12.72	1.38

Table III. The activity of several enzymes per 100 g body weight and per g liver tissue before and after starvation

	μmole substrate (100 g b.w.$^{-1}$ min^{-1})			μmole substrate (g liver^{-1} min^{-1})		
	A	B	C	D	E	F
	During carbohydrate rich diet	After 24 h of starvation	(B/A) × 100 %	During carbohydrate rich diet	After 24 h of starvation	(E/D) × 100 %
Glycogen synthetase I	2.9	2.4	83	0.80	1.1	137
Glycogen synthetase D	5.8	4.0	69	1.6	1.8	112
Phosphorylase a	38.2	30.1	79	10.5	14.0	133
Phosphorylase b	1.7	6.1	359	0.47	2.8	596
Hexokinase	1.9	1.3	68	0.52	0.60	115
Glucokinase	11.3	2.7	27	3.1	1.2	39
Glucose-6-phosphatase	37.1	43.5	117	10.2	19.8	194

Comparison of columns C and F clearly shows the large differences obtained with these units of enzyme activity.

and it decreases to 2–3% during 24 h of starvation. Table I shows that the water percentage is the same before and after starvation (69%). This means that the liver has lost about 50% of its water and dry material during 24 h of starvation, as the liver weight decreased about 50% during this period. During the diet approximately 50% of the dry material is due to glycogen. This has disappeared after starvation (Table I). Thus, a loss of 50% of the liver weight during 24 h of starvation can be explained by the disappearance of all the glycogen and the associated amount of water. Thus, liver weight largely depends on the glycogen percentage and this relation can be expressed in the formula: 31/(31-glycogen %) × liver weight at 0% glycogen.

The glycogen percentage and the liver weight can also increase very quickly. In rats fed with a carbohydrate-rich diet after 24 h of starvation we observed a linear increase of glycogen content with time, resulting in a 4.8% glycogen content within 4 h (Figure 1).

Different sizes of cells in glycogen-rich livers compared with livers depleted of glycogen can be expected. In livers containing 16.1% glycogen and 0% glycogen, liver weight was 2.12 and the cell diameter was 1.38 times larger in the former than in the latter (Table II). As the volume of a sphere is $^1/_6 \times$ (diameter)^3, this accounts for an increased volume or weight of 2.6. The difference between 2.6 and 2.1 can be readily explained by the fact that a cell is not exactly spherical.

The protein percentage is nearly doubled after starvation. This shows that the total amount of protein remains about the same. Consequently, the enzyme activity expressed per gram liver tissue also doubles. However, if enzyme activity is expressed per 100 g body weight, and total enzyme activity of the liver is constant, the measured enzyme activity will be about constant. These facts are illustrated in Figure 2 and Table III.

Zusammenfassung. Schwankungen des Glykogengehalts verursachen sehr schnelle Veränderungen des Lebergewichtes und daher auch solche des prozentualen Proteingehalts der Leber. Enzymaktivität sollte deshalb pro Körpergewicht und nicht pro Lebergewicht ausgedrückt werden, wie dies oft geschieht.

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