FRUCTOSE-INDUCED ALTERATIONS IN LIVER POLYSOME PROFILES AND Mg^{2+} LEVELS *

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1. Introduction

As has been shown by us [1, 2] and by others [3–5], intravenous or intraperitoneal injection of large amounts of fructose rapidly depletes liver adenosine triphosphate and inorganic phosphate in laboratory animals and human individuals. This probably interferes with many kinds of energy-requiring processes in the liver. We have previously shown that incorporation of ^{14}C-leucine into liver protein is transiently but strongly inhibited by intravenous administration of 5 mmoles per kg of D-fructose to rats [1]. The inhibition is maximal 2 to 5 min after the fructose injection and is practically over 25 min later. The inhibition is closely correlated with concomitant alterations in liver adenosine triphosphate [1].

The data presented here indicates that liver polysome profiles are essentially unaltered 5 min after the fructose injection. At 15 to 30 min, however, a marked disaggregation of large polysomes takes place. Determination of Mg^{2+} levels in plasma and in liver, kidney and heart homogenates indicates that Mg^{2+} is selectively released from the liver (and, to some extent, from the kidney) to the blood. Changes in hepatic levels of free Mg^{2+} are thought to be involved in the alterations of the polysome profiles.

2. Materials and methods

The test animals were female albino rats weighing 180 to 200 g. They were fasted overnight prior to the experiments. Liver ribosomes were isolated at various times relative to an intravenous injection of 5 mmoles per kg of D-fructose into the inferior vena cava exposed to laparatomy under pentobarbital anesthesia. Samples of liver were rapidly removed, weighed, and homogenized in cold Tris buffer (25 mM Tris, 25 mM NaCl, and 5 mM $MgCl_2$) containing 0.14 M sucrose and 100 μg/ml of heparin, using seven strokes of a Potter–Elvehjem type homogenizer. Deoxycholate and Triton X-100 were added from a 5% stock solution in distilled water to a final concentration of 1% for each, and the tissue was homogenized with two more strokes [6]. The resulting 10% liver homogenate was centrifuged for 5 min at 30,000 g. Supernatant (0.2 ml) was layered over a 0.5 – 1.5 M linear sucrose gradient made in the above buffer plus 40 μg/ml of heparin and centrifuged for 60 min at 192,000 g_{max} in the SW 50.1 rotor. The gradients were analyzed by passing them though a 2 mm Gilford flow cell connected to a Zeiss PMQ II recording spectrophotometer. Transmittance at 260 nm was recorded and converted to absorbance units. These were multiplied by 5 to correct for the 2 mm light path of the flow cell.

Mg^{2+} and Ca^{2+} in plasma and Mg^{2+} in liver, kidney, and heart homogenates were determined with a Varian Techtron Model 1000 atomic absorption spectrophotometer at appropriate dilutions. Total tissue homogenates were made in doubly distilled water with deoxycholate and Triton X-100 added as above. After a 5 min centrifugation at 30,000 g, the supernatant was used for Mg^{2+} determinations. Liver

* Part of this study was presented at the Symposium on Clinical and Metabolic Aspects of Fructose, Helsinki, January 1972 [17].

soluble cytosolic and microsomal fractions were prepared by homogenizing a weighed sample of liver in 5 vol of Tris buffer (25 mM Tris-HCl, pH 7.5, and 25 mM KCl) containing 0.25 M sucrose. After seven strokes of the Potter–Elvehjem homogenizer and a 10 min centrifugation at 8,000 *g*, a portion of the supernatant corresponding to 1 g of liver fresh weight was centrifuged for 2 hr at 105,000 *g*. The middle 1/3 of the supernatant was removed with a Pasteur pipette for Mg^{2+} determinations. The centrifuge tube was drained and the microsomal pellet was suspended and homogenized in 1% deoxycholate plus 1% Triton X-100 for Mg^{2+} determinations.

3. Results and discussion

We have previously shown that the incorporation of ^{14}C-leucine into liver protein is strongly inhibited 2 to 5 min after intravenous fructose administration to less than 10% of the control value [1]. The fructose effect is brief and by 30 min the value is again at the initial level. Comparison of the time courses of the changes in ATP levels and ^{14}C-leucine incorporation indicates a good correlation between these two variables [1]. In other test systems, the level of intracellular ATP and incorporation of radioactive amino acid into protein show an analogous correlation, although the time course may differ depending on the method used to alter the ATP level [7–9].

The sedimentation characteristics of liver total polysomal population before and at various times relative to the fructose injection are shown in fig. 1. The dose was identical to that used previously when ^{14}C-leucine incorporation was measured [1]. The size distribution of the ribosomes remains essentially unchanged at the time of the maximal inhibition of ^{14}C-leucine incorporation probably indicating an impaired aminoacylation. At 15 and 30 min, however, a marked gradual disaggregation of the large polysomes into monosomes is observed which suggests that the ribosomes are incapable of initiating a new cycle of peptide synthesis. Although the precise relationship between fructose administration and altered liver polysome profiles remains to be determined, changes in intracellular free Mg^{2+} levels may be involved.

The data in table 1 indicate that after fructose administration a significant increase of Mg^{2+} takes place in plasma. The increase is even more marked when the Mg^{2+} values are expressed relative to plasma Ca^{2+} or protein. These ratios are used to correct for the dilution effect due to the injection of different test compounds.

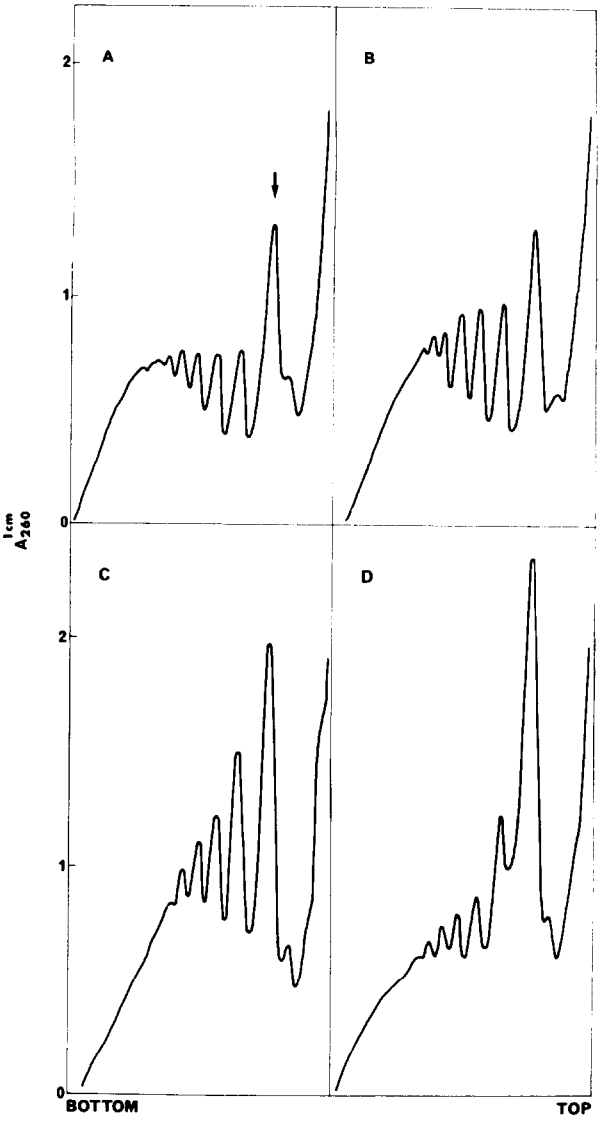

Fig. 1. Polysome profiles from rat liver (A) before, (B) 5 min, (C) 15 min, (D) 30 min after an intravenous injection of 5 mmoles per kg of D-fructose. Polysomes were analyzed and centrifuged as described in the text. Direction of sedimentation in the sucrose gradient is from right to left. Location of the monosome peak is indicated by an arrow in (A).

Table 1
Plasma Mg^{2+} levels 30 min after an intravenous injection of different test compounds.

Compound injected	No. of experiments	Plasma Mg^{2+}		
		mM	Mg^{2+}/Ca^{2+}	Mg^{2+}/protein (nmoles/mg)
None	7	0.74 ± 0.03	0.30 ± 0.02	15.4 ± 0.7
D-fructose	6	0.85 ± 0.03 †	0.41 ± 0.01 *	23.2 ± 0.8 *
D-glucose	5	0.72 ± 0.03	0.33 ± 0.01	15.8 ± 0.3
Saline	5	0.79 ± 0.02	0.36 ± 0.02	16.5 ± 0.4

5 mmoles per kg of fructose, or glucose, or an equivalent volume of saline was injected into the inferior vena cava under pentobarbital anesthesia. For further experimental details, see text. Values are means ± S.E., † = $P < 0.02$, * = $P < 0.01$, as compared to None value.

Table 2
Mg^{2+} in total liver homogenate, in 100,000 *g* supernatant, and in microsomal fraction 30 min after an intravenous injection of of different test compounds.

Compound injected	No. of experiments	Mg^{2+} (μmoles/g liver fresh weight)		
		Total homogenate	100,000 *g* supernatant	Microsomal fraction
None	6	9.03 ± 0.17	9.66 ± 0.03	13.30 ± 0.04
D-fructose	5	7.99 ± 0.02 *	8.41 ± 0.02 *	12.15 ± 0.06 †
D-glucose	5	9.38 ± 0.02	9.52 ± 0.01	13.55 ± 0.06
Saline	4	8.94 ± 0.02	9.49 ± 0.02	13.95 ± 0.05

Experimental conditions etc. as in table 1.

Table 3
Mg^{2+} in kidney and heart homogenates 30 min after an intravenous injection of different test compounds.

Compound injected	No. of experiments	Mg^{2+} (μmoles/g tissue fresh weight)	
		Kidney	Heart
None	7	8.01 ± 0.12	8.38 ± 0.09
D-fructose	5	7.21 ± 0.18 *	8.27 ± 0.09
D-glucose	5	8.51 ± 0.30	8.73 ± 0.48
Saline	5	7.94 ± 0.19	8.41 ± 0.17

Experimental conditions etc. as in table 1.

Mg^{2+} determinations in total liver, kidney, and heart homogenates indicate that injection of fructose results in a selective loss of Mg^{2+} from the liver (and, to some extent, from the kidney) to the blood (tables 2 and 3). We have previously shown that, in addition to the changes in liver ATP content, a moderate depression of ATP is also induced in the kidney by D-fructose [2]. This reflects the activity of fructokinase in these tissues [10]. In heart, however, this enzyme is absent and no changes in ATP [2] or Mg^{2+} (table 3) were observed. The control compounds, an equimolar amount of D-glucose and an equivalent volume of saline, did not significantly alter the Mg^{2+} levels in any of the test situations.

Protein synthesis in the cytoplasm of eukaryotic cells takes place on both free and membrane-bound polyribosomes. A number of reports have provided evidence that separate functions exist for the two populations of polyribosomes. Therefore, it was of interest to examine the changes in Mg^{2+} levels in soluble cytosolic and microsomal fractions separately. The results in table 2 show that, in both these liver subfractions, a significant decrease of Mg^{2+} takes place after fructose administration.

A large part of the Mg^{2+} present in the cell is bound to ATP. Due to the high association constant [11], as much as 30 to 40% of intracellular Mg^{2+} may be normally bound to ATP, assuming that there is no competition by other cations. Fructose injection results in, within a few minutes from the injection, a decrease of liver ATP to about 40% of the initial value [1]. Subsequently, part of the intracellular Mg^{2+} is released to the interstitial fluid and blood as demonstrated in this study. A related phenomenon may be an increase in serum Mg^{2+} documented in a patient with hereditary intolerance to fructose [12].

When the rebound of the liver ATP takes place by 30 min, the newly formed ATP again binds part of the available Mg^{2+}. This may lower the intracellular free Mg^{2+} level even more. Thus, the situation after fructose administration may bear a resemblance to that demonstrated *in vitro* when so-called runoff ribosomes are released from polyribosomes by incubation under conditions of amino acid incorporation with less than 7 mM Mg^{2+} [13, 14].

In this study, the total ribosomal population was examined. It can be further proposed that administration of fructose may influence the distribution of ribosomes between free and membrane-bound forms, since Mg^{2+} is thought to play an important role in the ribosome–membrane interaction [15]. A recent ultrastructural study supports this view [16].

Besides being a convenient *in vivo* system for studies on translational control mechanisms of eukaryotic protein synthesis, the findings, on the other hand, indicate the need for caution when large doses of intravenous fructose are used for various reasons in man.

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