Dietary sugar promotes gene activation in intestinal cell chromatin of adult rats[1]

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Summary. The effect of dietary sucrose on transcription activity of chromatin from intestinal cells of adult rats was investigated. Chromatin-bound RNA polymerase II activity was significantly enhanced after feeding sucrose to rats. RNA polymerase II transcribes the genes coding for pre-messenger RNA. The results indicate that feeding of sucrose increased the synthesis of pre-messenger RNA molecules coding for specific proteins which are involved in the adaptive processes of sucrose hydrolysis in intestinal cells.

Sucrose is known to promote enzyme adaptation in intestinal cells[2]. This process leads to the stimulation of sucrase synthesis[3,4]. Indirect evidence suggests that the increase in sucrase activity is regulated at the level of gene transcription[5,6]. In eukaryotic cells, 3 types of RNA polymerases are responsible for the synthesis of RNA. RNA polymerase I is associated with the nucleolus and produces pre-ribosomal RNA. Enzyme II is located in the nucleoplasm and synthesizes pre-messenger RNA. A similar location is found for RNA polymerase III which produces low molecular weight RNAs[7]. The RNA polymerases appear in the nuclei as chromatin-bound and free enzymes[8]. The chromatin-bound enzymes will continue to synthesize in vitro the in vivo initiated RNA chains. It has been shown previously that dietary proteins affect transcription of DNA to RNA in liver[9-11]. No publication has appeared as to the effect of dietary carbohydrates alone on gene transcription. We report here the results obtained on transcription activity in the chromatin of intestinal cells after feeding of sucrose to adult rats.

Materials and methods. Male rats (300 g) of the Sprague-Dawley strain were separated into 3 groups. Each group consisted of 6 rats. The 1st group was starved for 63 h. A 2nd group was starved for 48 h and thereafter had access to a solution of 70% sucrose during 15 h. Each rat consumed 7–10 ml of the sucrose solution. A 3rd group was fed ad libitum laboratory chow pellets. All animals had free access to water. One rat was used for each preparation. The rats were killed and a 40-cm segment of jejunum removed, washed with cold saline and everted. The cells were scraped off and homogenized in 1.75 M sucrose-1 mM $CaCl_2$. The suspension was centrifuged for 60 min at 63,500 × g_{av} in an SW 25 Beckman rotor. The nuclear pellet was suspended in 1 mM dithiothreitol, 4% glycerol and 1 mM Tris-HCl buffer (pH 8 measured at 25 °C). The nuclei were lyzed for 20 min at 4 °C[12]. The suspension was centrifuged at 5000 × g_{av} for 15 min. The chromatin pellet was suspended in the above medium to give an A_{260} of 20 OD units/ml.

The assay for chromatin-bound RNA polymerase activity was as follows. The final incubation volume was 130 µl. The mixture contained 30 mM Tris-HCl buffer, pH 7.8 at 25 °C, 2 mM $MgCl_2$, 2 mM $MnCl_2$, 1 mM dithiothreitol, 70 mM $(NH_4)_2SO_4$, 4% glycerol, 0.3 mM of each ATP, GTP, CTP, and 0.046 mM (^{3}H)UTP (250 mCi/mmol), 6 mM phosphocreatine, 0.50 U creatine phosphokinase. Where appropriate, 1.4 µg of α-amanitin/ml and/or 1 mg/ml of heparin were included. Approximately 1.6 OD units of the chromatin fraction was added.

After incubation for 10 min at 35 °C a 10% (w/v) solution of trichloroacetic acid-1 mM AMP (2 ml) was added. The precipitates were collected on glass-fiber filters, the filters rinsed with 5% (w/v) trichloroacetic acid and dried. Radioactivity was determined in a liquid scintillation spectrometer. Activity of RNA polymerase I and III was measured in the presence of a low concentration of α-amanitin. Activity of enzyme II was estimated from the difference between total and I plus III activity. RNA polymerase I and III were not distinguished here and were referred to as enzyme I activity.

Results and discussion. Animals were starved for 48 h and then given a 70% sucrose solution 15 h before sacrifice. Comparison was made with rats starved for 63 h and with animals fed laboratory chow pellets ad libitum. RNA polymerase activity was measured in chromatin of nuclei isolated from cells of the jejunal mucosa. The chromatin used in the experiments had a sedimentation value of more than 100 S_{20} which corresponds to the large domains of DNA containing more than 30 nucleosomes in their nucleomeric organization.

The results were related either to mg of DNA present in the chromatin fraction or to the DNA of the whole nuclei. In both reference systems the specific activity of RNA polymerase I was significantly reduced after 63 h of starvation as compared with the ad libitum fed rats (table 1). Results similar to ours on the effect of starvation have been published for the liver of rats[13] and mice[14]. After feeding of sucrose to the starved rats a significant (1.6-fold) increase in activity of RNA polymerase I was observed. The level was still significantly below that of the ad libitum fed rats. The results suggest that sucrose alone is insufficient to reactivate existing RNA polymerase I molecules. Alternatively, the enzyme molecules had been degraded during the starvation period and had not yet been replaced. It has been shown that the concentration of RNA polymerase I is responsible for a high rate of transcription under normal steady-state conditions[15]. Ad libitum feeding of our rats would correspond to such a physiological condition.

The activity of RNA polymerase II remained unaffected by starvation. In mouse kidney no decrease in activity of RNA polymerase II was observed after 40 h of starvation[14]. Feeding of sucrose to the starved rats provoked a stimulation of activity to a level significantly (1.3-fold) higher than that of the ad

Table 1. Effect of diet on chromatin-bound type I and type II RNA polymerase activities in rat intestinal cells

Animals	pmoles UMP incorporated into RNA Type I RNA polymerase per mg of chromatin DNA	per mg of nuclear DNA	Type II RNA polymerase per mg of chromatin DNA	per mg of nuclear DNA
Fed ad libitum	153.73 ± 20.00[a]	85.90 ± 9.94[a]	76.95 ± 3.89[a]	43.56 ± 1.71[a]
Starved	38.47 ± 3.20[b]	25.23 ± 2.77[b]	63.59 ± 2.80[a]	41.23 ± 2.63[a]
Starved followed by sucrose feeding	60.96 ± 3.17[c]	39.52 ± 2.58[c]	99.77 ± 7.53[b]	58.89 ± 4.39[b]

The results are the mean ± SEM of 6 rats per dietary group with duplicate incubations in each experiment. Significance of the difference between corresponding groups was calculated using Student's t-test. Data sharing a common superscript letter within one column are not significantly different; otherwise $p < 0.01$.

libitum fed rats. The enhanced activity of RNA polymerase II after feeding of sucrose indicates an increased synthesis of pre-messenger RNA molecules coding for specific proteins involved in the adaptive processes promoted by dietary sucrose. The sulphated polysaccharide heparin when added to chromatin will dissociate some of the proteins and facilitate transcription of DNA by previously blocked RNA polymerase II molecules[16]. The data on heparin-stimulated activity presented in table 2 are the differences between the results obtained in the absence and presence of heparin. The heparin-dependent activity was the same in the ad libitum fed and starved rats and similar to the non-heparin dependent activity. After sucrose feeding a 2-fold rise of the heparin-stimulated activity was observed. One interpretation of these data would be an increased number of inactive chromatin-bound RNA polymerase II molecules available when required. The observed stimulation by heparin after sucrose feeding may also be explained by an increased nucleotide elongation rate of RNA synthesis[16].

Sucrose induces the synthesis of sucrase necessary for the hydrolysis of disaccharides[3,4]. The high demand for the hydrolytic enzyme would require an increase in concentration of messenger RNA directing the synthesis of proteins. Our findings show that sucrose exerts a specific effect on gene activation, favouring the transcription of pre-messenger RNA genes by RNA polymerase II rather than the transcription of pre-ribosomal RNA genes by RNA polymerase I.

Table 2. Effect of dietary conditions on heparin-stimulated chromatin-bound type II RNA polymerase activity

Animals	pmoles UMP incorporated into RNA per mg of chromatin DNA	mg of nuclear DNA
Fed ad libitum	72.11 ± 6.12^a	40.72 ± 3.10^a
Starved	75.74 ± 7.11^a	48.60 ± 8.20^a
Starved followed by sucrose feeding	156.10 ± 6.48^b	104.56 ± 3.78^b

Heparin 1 mg/ml was included in the incubation mixtures. The results are the mean ± SEM of 6 rats per dietary group with duplicate incubations in each experiment. Data sharing a common superscript letter within one column are not significantly different; otherwise $p < 0.01$.

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Mitochondrial and cytosolic glutathione after depletion by phorone in isolated hepatocytes[1]

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Summary. The glutathione content of cytosol and mitochondria of isolated hepatocytes was depleted by addition of a low concentration of phorone (0.5 mM) by 75% and 40% respectively. Different rates of replenishment indicate metabolic separation of cytosolic and mitochondrial glutathione pools. The release from hepatocytes occurred at a rate of about 8 nmol/g wet weight/min, both in controls and after phorone depletion.

The presence of glutathione in liver mitochondria has been documented in the isolated organelles[3,4], in isolated hepatocytes[5] and in perfused liver[6]. The concentration of the mitochondrial glutathione reported previously ranges from 4.9 mM (0.2 μmol/g)[5] to 11 mM (0.45 μmol/g)[6], assuming 51 mg mitochondrial protein/g wet weight and 0.8 μl H_2O/mg mitochondrial protein[7]. The sizeable range is probably due to the different experimental models and fractionation procedures used.

The presence of more than one pool of glutathione in the liver was first suggested by Edwards and Westerfield[8] and has been confirmed more recently[9,10]. At variance with these observations, a recent study[11] showed kinetic homogeneity of the hepatic glutathione.

The latest approach to this problem[5] supported again the suggestion of 2 different pools of glutathione in isolated hepatocytes located mainly in cytosol and mitochondrion. In terms of incorporation of radio-labeled glutathione precursors, half-lives of 2 and 30 h were reported for the cytosolic and mitochondrial pools of glutathione, respectively.

The aim of this work was to assess levels of mitochondrial and cytosolic glutathione when synthesis of it takes place following depletion of the thiol.

Materials and methods. Animals. Male Wistar rats (180–200 g) fed on a stock diet (Altromin) were used for hepatocyte preparation. The experiments were started between 10.00 and 11.00 h to avoid changes in glutathione levels due to the circadian rhythm.

Preparation and incubation of isolated hepatocytes. Hepatocytes were isolated as described by Berry and Friend[12] with slight modifications[13]. For incubation, hepatocytes (approximately