lowed by photooxidation reactions[6,7]; c) finally, quenching could occur by a fast photo-induced conformational change (from a 'close' configuration to an 'open' one), which plays an important role in the phototherapy of hyperbilirubinemia[8,9].

A detailed study of fluorescence intensity and decay-time could help to determine the most effective quenching process and add new information on the excited state of BR.

1 Matheson, I.B.C., Faini, G.J., and Lee, J., Photochem. Photobiol. *21* (1975) 135.
2 Bonnett, R., Dalton, J., and Hamilton, D.E., J. chem. Soc. chem. Commun. *1975*, 639.
3 Cu, A., Bellah, G.C., and Lightner, D.A., J. Am. chem. Soc. *97* (1975) 2579.
4 Beaver, G.H., d'Albis, A., and Gratzer, W.B., Eur. J. Biochem. *33* (1973) 500.
5 Chen, R.F., Archs Biochem. Biophys. *160* (1974) 106.
6 Gijzeman, O.L.J., Faraday J. chem. Soc., II *70* (1974) 1143.
7 Mc Donagh, A.F., Biochem. biophys. Res. Commun. *44* (1971) 1306.
8 Mc Donagh, A.F., Lightner, D.A., and Wooldridge, A., J. chem. Soc. chem. Commun. *1979*, 110.
9 Schmid, R., Gastroenterology *74* (1978) 1307.

0014-4754/83/020165-02$1.50+0.20/0

Stimulation of intestinal chromatin template activity by dietary carbohydrates in adult rats

F. Raul and J.-F. Launay[1]

INSERM UNITE 61, 3, avenue Molière, F-67200 Strasbourg (France), April 13, 1982

Summary. Oral administration of a 70% solution of sucrose to starved adult rats resulted 1 h after feeding in a 3.5-fold stimulation of intestinal chromatin template activity assayed in vitro using *E. coli* RNA polymerase. A similar stimulatory effect was observed with fructose, whereas glucose exhibited a weaker effect, indicating that the nature of the ingested carbohydrate may have a direct effect on the extent of intestinal chromatin template activation.

Dietary carbohydrates have been shown to induce intestinal enzyme adaptation, sucrose or fructose exerting a specific stimulatory effect on disaccharidase activities[2-5]. Previous studies have demonstrated in rats that sucrose ingestion provoked the stimulation of sucrase activity along the villus-crypt axis[6-9], maximum stimulation occurring in the immature cells of the upper crypt zone and of the villus base[6,7]. Furthermore, the increase in sucrase activity was associated with enhanced sucrase synthesis in these cells[7,9]. In addition, it has been demonstrated that inhibition of RNA synthesis suppressed the stimulatory effect of sucrose feeding on sucrase or maltase activities[5,10] whereas other brush border enzymes (lactase and aminopeptidase) were not modified[10]. From these results it may be suggested that dietary sucrose could regulate transcription in the intestinal epithelial cell. The present study was undertaken in order to determine whether dietary carbohydrates have a direct effect on intestinal chromatin template activity and whether this effect is related to the nature of the ingested carbohydrate.

Materials and methods. Adult Wistar rats were housed in individual metabolic cages and starved for 72 h. After this period the animals received 5 ml of a 70% solution of carbohydrate (sucrose or fructose or glucose) by gavage; the controls received water or a 70% solution of mannitol. Nuclei were purified from proximal jejunum mucosa (10 cm length) or from liver (3 g) according to the method of Haussler et al.[11]. 20% mucosal homogenates (W/V) were made in 0.25 M sucrose, 0.05 M Tris HCl, pH 7.4, 0.025 M KCl and 0.005 M $MgCl_2$ (TKM) and centrifuged at 1200×g for 10 min to pellet crude nuclei. The crude nuclear pellet was then resuspended in 1.7 M sucrose in TKM to yield a 20% homogenate (W/V). Purified nuclei were harvested by centrifugation at 27,000×g for 20 min. Chromatin was prepared from purified nuclei as described by Brumbaugh and Haussler[12] and suspended in TKM to give a final DNA concentration of 15–20 µg/25 µl. This procedure yielded 37% recovery of total mucosa DNA. The assay for in vivo chromatin template activity was performed according to Zerwekh et al.[13]. The standard reaction mixture for nuclear chromatin template activity contained in a final vol. of 240 µl: 12 µmoles of Tris-HCl (pH 7.9); 0.75 µmoles of NaF: 0.075 µmoles each of GTP, CTP and ATP; 0.0125 µmoles of unlabeled UTP; 1 µCi of [5,6-^{3}H] UTP (62 Ci/mmoles, Amersham, U.K.) and 0.2 µmoles of $MgCl_2$, α-amanitin was added to ½ of the reaction mixture (0.1 µg/assay) and each reaction was started by the addition of 10 µl of *E. coli* polymerase (850 units/mg of protein, Sigma type III) and 25 µl of DNA (15–20 µg) as chromatin. Bacterial RNA polymerase was

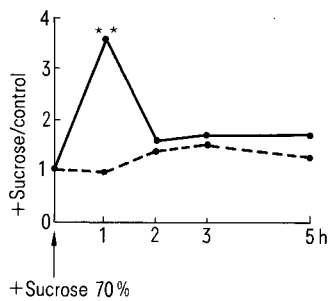

Figure 1. Time course of liver (---) and intestinal (—) mucosa cell chromatin template activation by sucrose administered in vivo. 5 ml of a 70% solution of sucrose was administered by gavage with a feeding needle to each rat previously starved for 72 h and a 70% solution of mannitol was administered to each control animal for the times indicated. Mannitol was used instead of water in order to ascertain that modification in chromatin template activity did not result from variations in osmotic pressure. The results are expressed as the ratio of template activity in the sucrose-treated rats to that in the controls. 6 animals per control and experimental group were assayed, each in triplicate, to obtain the template activity value at a given time. ** $p < 0.01$ (Student's t-test).

omitted from those assays with α-amanitin. Transcription was allowed to proceed for 10 min at 37 °C and was linear during the entire incubation period. Assays were terminated by pipetting the reaction mixture onto Whatman No. 3 MM filters which were washed successively in 10%, 5% trichloroacetic acid and in 95% ethanol. The cpm incorporated in the reactions containing α-amanitin represented transcription by endogenous RNA polymerases I and III. The cpm obtained from this control were tracted from those without α-amanitin to yield the *E. coli* RNA polymerase supported template activity. Endogenous RNA polymerases activities comprised in our conditions less than 0.8% of the total *E. coli* RNA polymerase-supported template activity, and showed no modulatory changes between control and experimental preparations.

Results and conclusions. In the present study bacterial RNA polymerase was used to estimate the extent of template activation. The limitation of this procedure is that the use of bacterial RNA polymerase could yield measurements of template activity that are not biologically relevant, and that homologous rat enzyme might be ideal for the template assays. However, as has been extensively discussed by others[13], *E. coli* RNA polymerase appears to be an acceptable substitute. It must be emphasized that the in vitro initiation sites on chromatin preparations may be different from those that are operative in the intact intestinal nuclei, but it is also likely that the extent of stimulation of chromatin template activity measured under the various experimental conditions will reflect directly a modification in the state of chromatin in vivo and therefore will be of physiological significance since the procedure used for chromatin preparation was identical in all conditions.

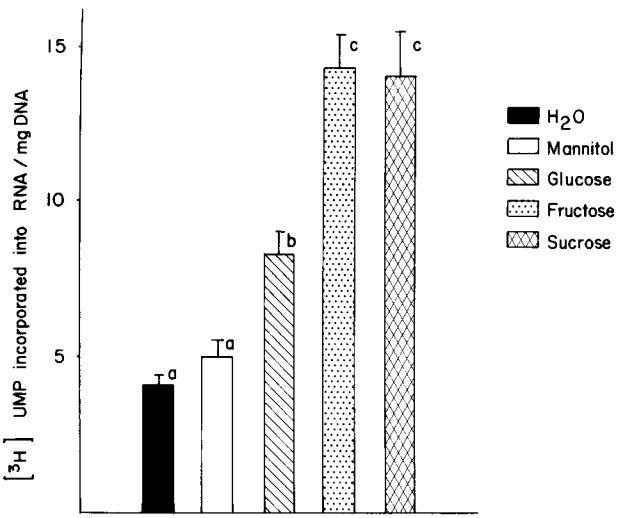

Figure 2. Intestinal chromatin template activity measured 1 h after oral administration of 5 ml of water or of a 70% solution of mannitol, glucose, fructose or sucrose to rats previously starved for 72 h. The results are expressed relative to the DNA concentration as determined on identical chromatin aliquots. Each column represents the mean value ±SE of 5 animals. For each condition, columns not sharing a common superscript letter differ significantly ($p < 0.01$ Student's t-test).

The time course of intestinal and liver chromatin template activity is shown in figure 1. The results are expressed as the ratio of template activity of the sucrose-fed animals to that of controls receiving mannitol. The average values (nmole of UMP/mg of DNA) in the intestine for the controls receiving mannitol did not exhibit any significant change during the various time periods when compared to the starting value at time 0, which was 3.91 ± 0.24. In contrast, a significant stimulation (3.5 times) of intestinal chromatin template activity was seen 1 h after sucrose feeding, reaching a value of 14.09 ± 2.80. Thereafter the template activity dropped to a plateau at a level not significantly different from the starting value. Liver chromatin template activity did not exhibit any modulatory changes during the time-course study when compared to the starting value, which was 10.21 ± 1.51. As shown in figure 2, the nature of the carbohydrate has a direct influence on the stimulation of intestinal chromatin template activity measured 1 h after feeding; as shown above, this time corresponds to maximum stimulation of template activity after sucrose feeding. The animals receiving mannitol showed no significant variation in intestinal chromatin template activity when compared to those receiving water, with values reaching respectively 5.06×0.35 and 4.14 ± 0.24. Intestinal chromatin template activity was significantly enhanced by glucose up to a value of 8.25 ± 0.75. However, maximum stimulation was measured after sucrose or fructose ingestion, template activity reaching values respectively of 13.98 ± 2.52 and 14.34 ± 1.29.

These results showed a stimulation in intestinal chromatin template activity by dietary sucrose or fructose. This might have physiological importance since it has been shown that dietary fructose and sucrose induce enzyme adaptation in the intestine, fructose being in this process the most active part of the sucrose molecule[4,14]. Our findings provide additional evidence in support of the hypothesis that fructose or sucrose might stimulate enzyme activity by acting at the level of transcription in the intestinal epithelial cell.

1 The authors like to thank F. Gossé and M. Galluser for excellent technical assistance. This work was supported by DGRST grant No. 81 L 1312.

2 Blair, D.G.R., Yakimets, W., and Tuba, J., Can. J. Biochem. *41* (1963) 917.
3 Deren, J.J., Broitman, S.A., and Zamcheck, N., J. clin. Invest. *46* (1967) 186.
4 Rosensweig, N.S., and Herman, R.H., J. clin. Invest. *47* (1968) 2253.
5 Kimura, T., Seto, A., and Yoshida, A., J. Nutr. *108* (1978) 1087.
6 Ulshen, M.M., and Grand, R.J., J. clin. Invest. *64* (1979) 1097.
7 Raul, F., Simon, P.M., Kedinger, M., Grenier, J.F., and Haffen, K., Biochim. biophys. Acta *1* (1980) 630.
8 Yamada, K., Bustamante, S., and Koldovsky, O., FEBS Lett. *129* (1981) 89.
9 Cezard, J.P., Broyart, J.P., Cuisinier-Gleizes, P., and Mathieu, H., Gastroenterology *80* (1981) 1121.
10 Raul, F., Pousse, A., Grenier, J.F., and Haffen, K., Gastroent. clin. Biol. *6* (1982) 424.
11 Haussler, M.R., Myrtle, J.F., and Norman, A.W., J. biol. Chem. *243* (1968) 4055.
12 Brumbaugh, P.F., and Haussler, M.R., J. biol. Chem. *249* (1974) 1251.
13 Zerwekh, J.E., Lindell, T.J., and Haussler, M.R., J. biol. Chem. *251* (1976) 2388.
14 Raul, F., Kedinger, M., Simon, P.M., Grenier, J.F., and Haffen, K., Biol. Neonate *39* (1981) 200.

0014-4754/83/020166-02$1.50 + 0.20/0