Table 1. Effect of injection of L-cysteine on concentration of reduced glutathione (GSH) in rat brain

Dose of cysteine (g/kg b.wt)	GSH concentration (μmole/g fresh wt)	% of the controls
0.00	3.25 ± 0.38 (5)	100
0.25	2.04 ± 0.16 (3)*	63
0.50	1.95 ± 0.20 (3)*	60
1.00	1.90 ± 0.05 (3)*	58

Rats were injected (i.p.) with a 1 M solution of cysteine, and killed 2 h after injection. Results are means ± SD for the number of observations in parenthesis. * $p < 0.005$.

Table 2. Effect of injection of N-acetyl cysteine on concentration of reduced glutathione (GSH) in rat brain

Dose of N-acetyl cysteine (g/kg b.wt)	GSH concentration (μmole/g fresh wt)	% of the controls
0.00	3.25 ± 0.38 (5)	100
0.25	2.69 ± 0.18 (3)*	83
0.50	2.49 ± 0.29 (3)*	77
1.00	1.95 ± 0.15 (3)**	60

Rats were injected (i.p.) with a 1 M solution of N-acetyl cysteine, and killed 2 h after injection. Results are means ± SD for the number of observations in parentheses. * $p < 0.05$; ** $p < 0.005$.

It is well established that normal GSH concentrations are important to maintain plasma membrane integrity[7].

Mitchell et al.[8] showed that the liver necrosis induced by paracetamol (acetaminophen) overdose is due to GSH depletion. It is known that cysteine may be spontaneously oxidized to give cysteine disulphide and H_2O_2[9]. We have postulated[2] that the cysteine-induced GSH depletion in hepatocytes is due to formation of H_2O_2 with subsequent damage to cell membranes.

Olney et al.[5] suggested that a possible mechanism of cysteine induced cytotoxicity could be related to the conversion of L-cysteine to more acidic derivatives. However, the oxidation of cysteine to yield cystine and H_2O_2 is a much more rapid reaction, and hydrogen peroxide is known to be a very toxic substance, which could account for the widespread lesions observed after injection of cysteine, in contrast with the more localized lesions observed when rats are treated with acidic amino acids, i.e. glutamate. The cysteine derivative N-acetyl cysteine is commonly used as a mucolytic agent[10]. Thus, the fact that high doses of N-acetyl cysteine can also promote GSH depletion in brain should be considered.

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Fluorescence of free bilirubin at room temperature

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Summary. Very weak fluorescence from free bilirubin in liquid solvents at room temperature was clearly recorded. The emission band is peaked at about 540–550 nm and its width is about 75 nm.

Bilirubin (BR) is known to fluorescence at low temperature when in glassy solvents[1-3], and at room temperature when bound to albumins[4,5] and to micellar systems[3]. However, when free bilirubin is in liquid solvents at room temperature, fluorescence is uncertain and controversial[3-5]. Under these conditions emission quantum yields are, in fact, very low ($\leqslant 10^{-4}$) and conventional spectrofluorometers can generally give only ambiguous results. In order to obtain more precise results we used a laser-excited fluorometer with double monochromator and photon-counting electronics. Freshly prepared solutions of purified BR in N-N′-dimethylformamide, chloroform, carbon tetrachloride and water (0.1 N NaOH added) were irradiated with an unfocused Argon laser beam (1–5 mW) and emission spectra were recorded for only a few minutes in order to minimize photo-oxidation processes. In all cases an asymmetrical emission band was observed, with a half-height width of about 2500 cm^{-1} (75 nm) and the intensity peak at about 18,300 cm^{-1} (545 nm) corresponding to a large Stokes shift of about 3600 cm^{-1} (100 nm). Emission intensity was proportional to BR concentration up to saturation and relative quantum yield depended upon solvents: dimethylformamide > chloroform > water (pH 10) > carbon tetrachloride. In basic aqueous solutions sharp peaks were observed on the emission background and they can be attributed to Resonance Raman bands of the BR chromophore, occurring at 1280, 1350, 1590 and 1620 cm^{-1} (Raman shifts).

The actual mechanism of fluorescence quenching is not known but some suggestions can be made: a) collisions of free BR with solvent molecules inducing direct nonradiative decay could be an important quenching mechanism, in agreement with the strong fluorescence enhancement for BR bound to proteins or in rigid solutions; b) another possible quenching process involves specific collisions with oxygen, promoting an efficient intersystem crossing, fol-

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.

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

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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

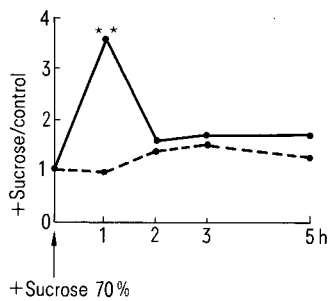

Figure 1. Time course of liver (---) and intestinal (—) mucosa cell chromatin template activation by sucrose administered in vivo. 5 ml of a 70% solution of sucrose was administered by gavage with a feeding needle to each rat previously starved for 72 h and a 70% solution of mannitol was administered 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).