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 $p < 0.01$.

1 Acknowledgments. This work was supported in part by the Swedish Medical Research Council and I.N.S.E.R.M. (France). F.R. was supported by a postdoctoral fellowship of the Swedish Medical Research Council (Project No. K82-13F-6371-01) within the scientific exchange program between Sweden and France.

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0014-4754/84/040364-02$1.50 + 0.20/0

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

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2 August 1983

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

10 mg protein/ml) were transferred into 25-ml conical flasks containing incubation medium at 37 °C, equilibrated with a gas mixture of O_2/CO_2 (19:1, v/v). Final concentrations were: glucose, 10 mM; L-lactate, 2.1 mM; pyruvate, 0.3 mM; DL-3-hydroxybutyrate, 0.6 mM; acetoacetate, 0.3 mM; the salt mixture described by Krebs and Henseleit[14], and, unless otherwise stated, glutamine, 5 mM; glycine, 5 mM; serine, 2 mM; and methionine, 1 mM.

Phorone (2,6-dimethyl-2,5-heptadiene-4-one) was dissolved, for the stock solution, in DMSO (dimethylsulfoxide) to a final concentration of 100 mM. Additions were made at the beginning of the incubation.

Separation and fractionation of the hepatocytes. The digitonin fractionation procedure[15] was used with the modifications as described[13].

The medium for the separation and fractionation of the cells consisted of sucrose, 0.25 M; MOPS (morpholinopropanesulphonic acid) 20 mM (pH 7.0); EDTA, 3 mM; and digitonin, 2 mM. Digitonin was absent during the separation of the cells from incubation medium. The exposure time after addition of hepatocyte suspension to the separation medium was 15 sec with or without digitonin being present. The pellet and supernatant fractions of the digitonin-treated cells were designated mitochondrial and cytosolic, respectively. In the absence of digitonin, the supernatant was designated extracellular.

The viability of hepatocytes was assessed by 2 parameters: lactate dehydrogenase leakage, as a marker of plasma membrane damage, and ATP/ADP ratio for the metabolic competence of the cells.

Assays. Glutathione was measured by the kinetic assay with DTNB (5,5'-dithiobis-nitrobenzoic acid) and glutathione reductase (E.C. 1.6.4.2.)[16]. The results are, in all cases, expressed as GSH equivalents (GSH + 2 GSSG) and referred to the total protein content of the incubation. The measurements of adenine nucleotides were performed according to Soboll et al.[17], in a Sigma ZWS 11 dual-wavelength spectrophotometer (Biochem. Co., München, FRG) at 340–400 nm. Lactate dehydrogenase activity in the extracellular samples was measured as previously described[18], and is expressed as the percentage of the total activity in the hepatocyte incubation mixture, obtained in the presence of 1% Triton X-100.

Chemicals and biochemicals. Phorone was purchased from Fluka (Neu-Ulm, FRG). Chemicals, biochemicals and enzymes were from Merck (Darmstadt, FRG), Boehringer (Mannheim, FRG) and Serva (Heidelberg, FRG).

Results. Glutathione depletion in isolated hepatocytes upon addition of phorone. Figure 1 shows the effect of different concentrations of phorone on the glutathione content of isolated hepatocytes. Maximal effect at all the concentrations tested was obtained within the first 30 min of incubation, whether the amino acids were present to allow glutathione synthesis or not. Phorone-elicited cellular depletion of glutathione does not proceed spontaneously. This is supported by the observation that

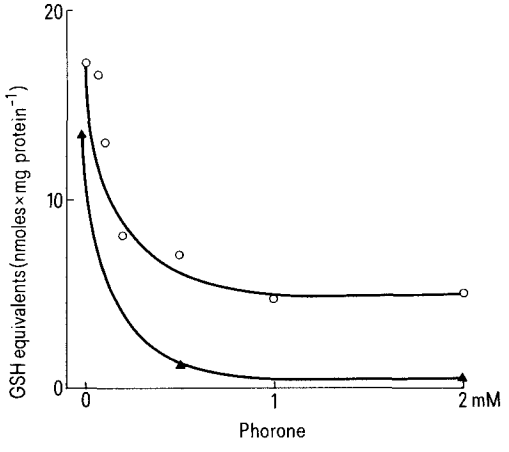

Figure 1. Effect of different concentrations of phorone on glutathione content of isolated hepatocytes after 30 min. ○, Content of the whole incubation mixture and, ▲, intracellular values. Conditions as in 'Material and methods' without amino acids.

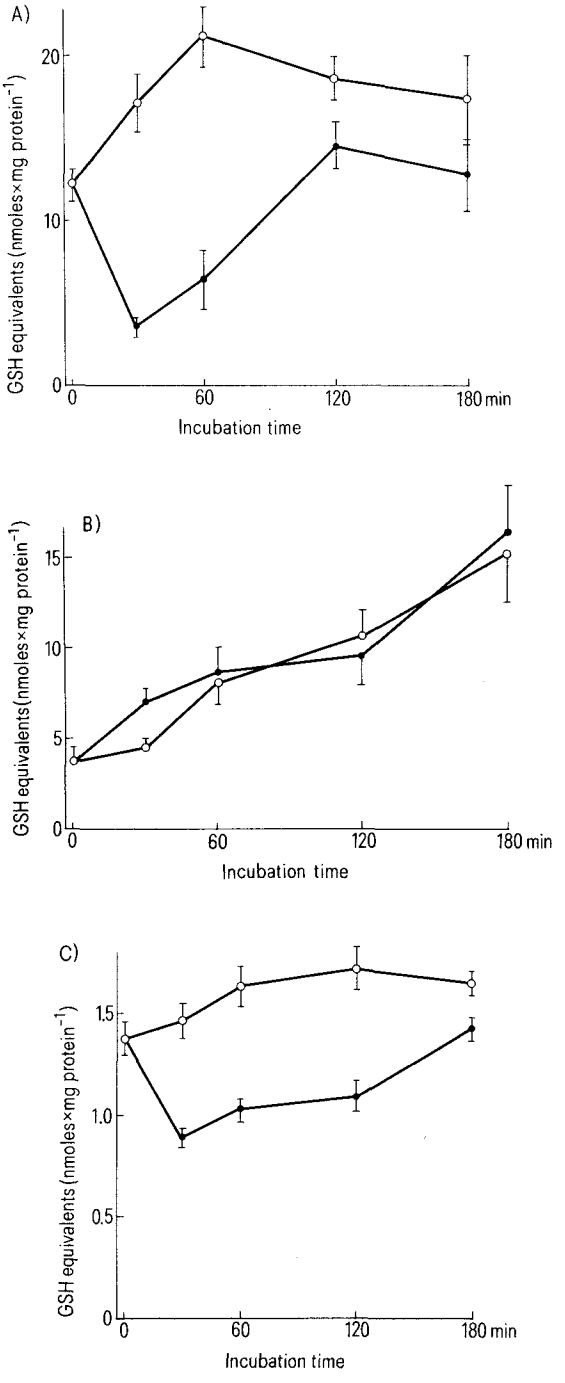

Figure 2. Time course of glutathione content in cytosol (A), extracellular space (B) and mitochondrial fractions (C), in the presence (●) and absence (○) of phorone 0.5 mM. Cells incubated as in 'Material and methods'. Results are means of 3–5 experiments with SEM represented by brackets.

the non-conjugated glutathione found at the end of the incubation was located outside the cell (fig. 1). Moreover, 2nd addition of phorone after 30 min of incubation, to exclude a possible lack of it after this period, did not promote a further depletion of glutathione (in the absence of amino acids), and also no spontaneous reaction between phorone and glutathione occurred in a cell-free system (data not shown).

The concentration of phorone chosen to perform the compartmental studies was 0.5 mM due to its nearly maximal effect and the lack of membrane damage, as measured by LDH leakage from the cells.

Cell viability during phorone incubation. Hepatocytes were not affected by phorone incubation as judged by the integrity of membrane permeability (LDH leakage) and ATP/ADP ratio. LDH activities in the extracellular space after 3 h of incubation were $12.8 \pm 1.6\%$ and $15.4 \pm 2.5\%$ in the control and phorone samples respectively. The ATP/ADP ratios were between 4.3 ± 0.5 and 6.2 ± 0.7 in the control and phorone treated cells and thus were similar to what was reported by others[19,20]. The addition of the corresponding amounts of the phorone solvent, DMSO, had no effect on LDH leakage, ATP/ADP ratio or glutathione content (data not shown).

Compartmentation of glutathione in isolated hepatocytes. The content of glutathione in the cytosol reached a steady value in the control within the 1st h and remained fairly constant up to the end of the experiment. In the presence of phorone, the cytosolic content was maximally depleted within the first 30 min of incubation and reached values similar to the control after 2 h (fig. 2A), but release into the extracellular space also occurred at a rate similar to the controls (fig. 2B). The release took place at a rate of 3.4 nmol/mg protein/h, which corresponds to 7.6 nmol/g wet wt/min, assuming 133 mg/g wet wt[7] for the calculation.

Figure 2C shows that the mitochondrial glutathione content can be partially restored following phorone-elicited depletion. From these results a rate of replenishment of 0.2 nmol/mg protein/h (0.4 nmol/g wet wt/min) can be calculated for the phorone-treated cells.

Discussion. Glutathione content can be depleted in cytosol and mitochondria upon addition of 0.5 mM phorone. This low concentration of phorone and the presence of methionine in the incubation medium were used to minimize a possible role of lipid peroxidation on cellular glutathione status or cell viability. Methionine was shown to prevent lipid peroxidation of isolated hepatocytes upon long-term incubation[21].

The phorone-elicited transient glutathione depletion did not affect the rate of release of it into the extracellular space (fig. 2B). The value of about 8 nmol/g wet wt/min obtained for the control and phorone-treated cells is in the range of those previously described[22,23].

As with ethacrynic acid[5] mitochondrial glutathione was not depleted by more than 40% of its initial content upon addition of phorone (fig. 2C) without affecting cell viability. The reason may reside in the fact that the fraction designated 'mitochondrial' obtained with the digitonin fractionation procedure contains the non-soluble material found in the cell (designated 'particulate' in Zuurendonck and Tager[15]). Therefore, glutathione in a bound form which may be acid-releasable or not accessible to the S-transferases, could be included in these samples and may be important in preventing cell damage. The results of figures 2A and 2C when expressed in relation to the controls (fig. 3) show a greater rate of replenishment for the cytosolic than for the mitochondrial glutathione.

The existence of a 'stable' pool of glutathione, i.e., a non-depletable pool by fasting or treatment with active metabolic compounds (di-butyryl-3′,5′-cyclic AMP), has been discussed[9,10] and recently attributed to the mitochondrial compartment[5]. The size of the mitochondrial pool of glutathione, of about 0.2 µmol/g wet wt as found here or of 0.45 µmol/g wet wt as found previously[6], however, cannot account for the stable pool (3 µmol/g wet wt)[9,10]. The location and metabolism of this resting glutathione, therefore, remains to be addressed.

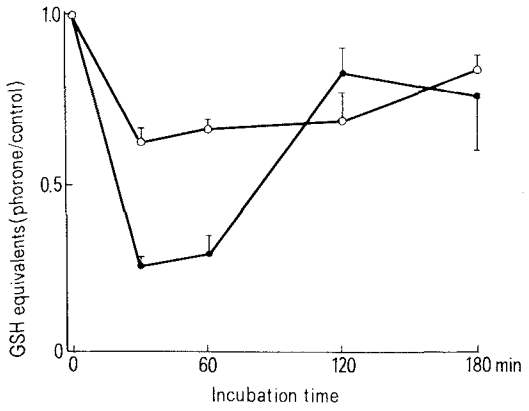

Figure 3. Time course of the ratio of glutathione contents in phorone and control samples, respectively; in cytosol (●) and mitochondria (○). Data are from figure 2.

1 This work was supported by the Ministerium für Wissenschaft und Forschung, Nordrhein-Westfalen, and by Deutsche Forschungsgemeinschaft.

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