The content of glutathione and glutathione S-transferases and the glutathione peroxidase activity in rat liver nuclei determined by a non-aqueous technique of cell fractionation

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Hepatocellular nuclei require glutathione, glutathione S-transferases (GSTs) and Se-dependent glutathione peroxidase (GPx) for intranuclear protection against damage from electrophiles or products of active oxygen. Data so far available from the literature on nuclei isolated in aqueous systems range from glutathione, GSTs and GPx either being absent altogether to being present in quantities in excess of those in the cytoplasm. This paper describes a small-scale preparation of a nuclear fraction from rat liver by a non-aqueous technique, designed to

retain nuclear water-soluble molecules *in situ*, since low-molecular-mass compounds can diffuse freely into other compartments during aqueous separation. This non-aqueous procedure shows the nucleus to contain glutathione at 8.4 mM and soluble GSTs at 38 µg/mg of protein, the enrichment over the homogenate being 1.2–1.4-fold. Se-dependent GPx activity was also present in the nucleus (56 m-units/mg), although with slightly lower activity than in the homogenate (0.7-fold).

INTRODUCTION

It is reasonable to suppose that glutathione (GSH) and the soluble glutathione S-transferases (GSTs) are present in liver nuclei in order to protect the genome and the numerous nuclear factors involved in gene expression from attack by genotoxic and cytotoxic electrophiles, resulting from the special function of the liver as an organ of xenobiotic metabolism [1,2]. Because of the permeability due to nuclear pores, it has been concluded that endogenous nuclear GSH levels cannot be determined by methods involving cell disruption in aqueous media [3]. However, in a study using isolated hepatocytes and monochlorobimane as an indicator of GSH *in vivo*, it was concluded that in the hepatocyte there is a nuclear/cytoplasmic GSH concentration gradient of approx. 3, maintained by an ATP-dependent mechanism [4]. This would mean that nuclear GSH would be present at approx. 20 mM concentration.

Conflicting views have also been published concerning the nuclear distribution of GSTs. The molecular mass of the GSTs is approx. 50000 Da, which is close to the limit proposed for exclusion by uptake through the nuclear pore [5]. Immunocytochemical analyses are not consistent. GSTs have shown to be either present in the nucleus [5] or absent [6–8]. When sucrose density gradients were used, the nuclear distribution of GSTs proved to be pH-dependent. In preparations that were obtained by using media at physiological pH [9], GSTs were absent from the nucleus [10]. However, when the preparation was carried out at pH 2.3 [11], nuclear GSTs occurred at high levels [10,12], apparently forming part of the non-histone chromatin protein fraction. When lactate dehydrogenase was incubated with nuclei isolated at pH 7.4 and the nuclei were subsequently isolated in the presence of citric acid as in [11] there was a clear association of the lactate dehydrogenase with the acid-treated nuclei [10].

Such aqueous cellular subfractionation techniques may therefore be of limited value.

A non-aqueous technique preventing the diffusion of aqueous solutes has previously been used to show that kidney cell nuclei contain similar amounts of GSH to those in the cytoplasm [13]. No results are available so far for hepatocyte nuclei. For the present work, we adapted a non-aqueous technique to small scale, based on the method described by Siebert [14], which yields a rat liver nuclear fraction suitable for the determination of the content of GSH, GSTs and glutathione peroxidase (GPx).

EXPERIMENTAL

Materials

Unless otherwise stated, enzymes and coenzymes were either from Boehringer (Mannheim, Germany) or from Sigma (Deisenhofen, Germany), and chemicals were from Merck (Darmstadt, Germany). Histones from calf thymus were from Sigma (St. Louis, MO, U.S.A.). Antisera for Alpha and Mu class glutathione S-transferases were obtained from Biotrin (Dublin, Ireland). ³⁵S-labelled Protein A was obtained from Amersham Radiochemicals (Amersham, U.K.).

Non-aqueous technique for the preparation of nuclear fractions on a small scale

The preparation of nuclear fractions uses a modification of the non-aqueous technique of ref. [14], as follows.

Homogenate

Two livers from male Wistar rats weighing 150–200 g, and

starved for 24 h, were isolated and perfused for 5–10 min with Krebs–Henseleit bicarbonate buffer equilibrated with O_2/CO_2 (19:1), freeze-clamped, ground in liquid nitrogen and freeze-dried at 0.26 Pa and $-40^\circ C$.

The dry liver powder (600 mg dry wt.) was divided into two portions, each of which was suspended in 11 ml of a mixture of heptane/ CCl_4 (H/C), relative density (d) 1.230 at $4^\circ C$, and sonicated at 5 s intervals of stop and sonication, respectively, for 4 min, then interrupted for 1 min, followed by another 4 min period of 5 s intervals of stop and sonication, so that the total sonication time amounted to 4 min. During the homogenization the suspension was continuously cooled in heptane/solid CO_2 . All subsequent steps were performed at $4^\circ C$. The resulting homogenates were filtered through a nylon mesh (pore size 200 μm) and the filtrate was agitated for about 2 h in glass vials filled with glass beads (4 mm diameter) to about two-thirds of the total volume. The homogenate was filtered again and samples were removed for analyses. The progress of both the homogenization and the subsequent fractionation was monitored by light microscopy. Approx. 5 μl of the suspension of a homogenate, or a fraction derived from it, was transferred to a glass slide, dried, and 200 μl of a 0.2% (w/v) aq. solution of Methylene Blue, a nuclear stain, was layered over the dry suspension.

Subfractionation

(1) The homogenate was centrifuged at 650 g for 3×30 s to remove any remaining large tissue fragments. The supernatant was then sedimented for 30 min at 2020 g and the pellet resuspended in H/C, $d = 1.315$. The resulting suspension was centrifuged for 1 h at 2020 g and the pellet washed with the same medium.

(2) The sediment from the above fractionation was suspended in H/C, $d = 1.330$, and centrifuged for 80 min at 2020 g . This step was repeated.

(3) The combined pellets were suspended in H/C, $d = 1.370$, and centrifuged for 1 h at 2020 g . The nuclei were then found in the supernatant, which was decanted, mixed with 1–3 ml of heptane and centrifuged for 30 min at 2020 g to sediment the nuclei. The resulting pellet was resuspended in H/C, $d = 1.330$, and centrifuged for 1 h at 2020 g .

(4) The pellet obtained was suspended in H/C, $d = 1.340$, and centrifuged for 1 h at 2020 g . The nuclei were recovered once more in the supernatant and were sedimented after dilution with 1–3 ml of heptane as described in step (3).

(5) The sediment was suspended in H/C, $d = 1.335$, and centrifuged for 1 h at 2020 g . This step was repeated once to give a nuclear fraction 80% pure and containing about 2 mg of protein. This amounts to about 5% of the theoretical yield of 42 mg of nuclear protein, assuming that 10% of the cellular protein is nuclear and that the dry-weight/protein ratio is 1.43 [15]. The main loss is due to nuclei with adhering cytosol which cannot be fractionated because of negligible differences in density compared with the other non-nuclear fractions. The yield could therefore be improved by longer homogenization. However, in this case the amount of broken nuclei would increase (see the Discussion section).

During the sedimentation procedure, a decision must be made, based on inspection under the light microscope, whether to proceed as described above after separation at H/C, $d = 1.370$, or to vary the density slightly. Sometimes the nuclei may be found in the $d = 1.340$ pellet rather than its supernatant. In this case, centrifugation in slightly heavier media is required, which brings the nuclei into the supernatant.

Electron microscopy

Dried samples from the nuclear fraction as well as from the homogenate were rehydrated in buffer (0.1 M cacodylate/HCl, 0.1 M sucrose, 2 mM $CaCl_2$, pH 7.4) fixed with 1% (w/v) glutaraldehyde in this buffer for 15 min at $4^\circ C$, post-fixed with 2% (w/v) OsO_4 in the same buffer for 45 min at $4^\circ C$, dehydrated in a graded ethanol series and embedded in Epoxy resin. Ultrathin sections were stained with uranyl acetate and lead citrate before examination in a Philips EM 400 electron microscope.

Biochemical markers for cell fractionation and enzyme activities

Samples obtained from the fractionation were dried over paraffin pellets *in vacuo* and suspended in 0.05 M phosphate buffer, pH 7.4, by shaking with quartz sand. DNA was determined as described in [16]. NAD pyrophosphorylase and citrate synthase activities were measured by modifications of the procedures described in [17] and [18] respectively. NADPH–cytochrome *c* reductase activity was determined as in [19], and GPx (t-butyl hydroperoxide as substrate) as in [20]. Protein was determined as in [21]. Histones were detected by SDS/PAGE [22], stained with Coomassie Blue R-250.

Determination of glutathione and soluble GSTs in cell fractions

Samples of homogenate and nuclear fractions were agitated in 0.15 M NaCl adjusted to pH 6.8 with 10 mM sodium phosphate, followed by centrifugation to give saline-soluble and saline-insoluble fractions. GST activity towards 1-chloro-2,4-dinitrobenzene was determined in the saline-soluble fractions by the method of Habig et al. [23] at $37^\circ C$. Both the saline-soluble and saline-insoluble fractions were analysed by SDS/PAGE stained with Coomassie Blue R250, using purified GST isoforms and histones as markers.

Although bands co-migrating with GST standards were clearly detectable on SDS/PAGE, quantification by densitometric analysis was not used, since it was possible that other unidentified proteins co-migrated with GSTs. Immunoquantification by Western blot was undertaken, and both (GSH + GSSG) and GSTs were determined on the same sample. Each fraction, containing approx. 1 mg of protein, was dispersed in 250 μl of a solution of 1% sodium deoxycholate in 50 mM sodium phosphate buffer, pH 7.4. Then 50 μl of 10% trichloroacetic acid was added, precipitating protein and nucleic acid to give a supernatant containing soluble low-molecular-mass components including glutathione. The supernatant was neutralized with 6 μl of triethanolamine and its total glutathione contents (GSH + GSSG) were determined [24]. The sediment was dissolved by boiling for 5 min in 1% SDS, and protein content was estimated as described in [25] with BSA as a standard. Samples containing 20–100 μg of protein were analysed by Western blotting using a modification of the method of [26]. After separation on SDS/PAGE, protein was transferred to an Immobilon P membrane (Millipore, Watford, Herts., U.K.). Unoccupied binding sites on the membrane were blocked by incubation with 50 mM Tris buffer/0.5 M NaCl/0.5% Tween 20 at pH 7.5, for 1 h, followed by washing with 20 mM Tris/0.5 M NaCl/0.05% Tween 20 (TTBS). The blots were then incubated for 2 h with either anti-GST 1-1 plus anti-GST 2-2, anti-GST 3-3 plus anti-GST 4-4 or anti-(microsomal GST). They were then washed several times with TTBS, incubated with 7.5 ml of ^{35}S -Protein A in 25 ml of TTBS for 2 h with shaking, washed with TTBS, air-dried and autoradiographed. The developed autoradiograph was used to locate GSTs on the blot (GST subunits 1, 2 and microsomal GST

each giving a separate bands, whereas subunits 3 and 4 overlap). Radiolabelled bands were excised and counted by liquid-scintillation spectrometry. Quantification was by comparison with standard GSTs separated on the same blot. Values were obtained in triplicate.

Soluble GST standards were from rat liver and were isolated by GST-agarose affinity chromatography [27]. Microsomal GST was a gift from Dr. Ralf Morgenstern (Karolinska Institute, Stockholm, Sweden).

RESULTS

Non-aqueous technique applied to hepatic nuclei

Light-microscopic as well as electron-microscopic analysis of the homogenate versus the nuclear fraction shows a considerable enrichment of nuclei over other cellular components. Electron micrographs reveal some contamination with mitochondria and other cytoplasmic material (results not shown).

DNA, NAD pyrophosphorylase and histones were used as nuclear markers, and mitochondrial citrate synthase, microsomal NADPH-cytochrome *c* reductase and microsomal GST were chosen as extranuclear markers (Table 1). Calculations based on these measurements show a 10% contamination with mitochondrial material (citrate synthase activity) and a 4% contamination with endoplasmic reticulum (represented by NADPH-cytochrome *c* reductase activity), assuming that 34% of the total cellular protein is mitochondrial and 10% is represented by the endoplasmic-reticulum compartment [15,19]. The content of DNA in the nuclear fraction is 7–9-fold greater than in the homogenate. Since the theoretical limit of nuclear enrichment is about 10-fold, assuming the hepatocyte nuclear volume to be 9.8% [28], it is concluded that the nuclear fraction obtained by this technique is approx. 80% pure. The lower enrichment of the nuclear marker enzyme NAD pyrophosphorylase (5–6-fold) may indicate that some nuclear protein from

broken nuclei with lower density co-sediments with lighter fractions.

The following considerations were found to be essential for preservation and successful fractionation of liver nuclei.

(i) Livers are from rats fasted overnight.

(ii) It is essential that the livers are well oxygenated at the time of freeze-clamping. Apparently the energy supply of the liver has an influence on the physical state of its nuclei.

(iii) The homogenization should be monitored carefully. If it is not complete, nuclei will be retained in partly broken cells and cannot be separated, but if it is too vigorous, nuclei will be fragmented.

Glutathione, GSTs and GPx

Values for total glutathione (GSH + GSSG), total soluble GST contents and GPx activity are also shown in Table 1. Glutathione has a similar distribution in both the nuclear fraction and the homogenate, the nuclear enrichment being about 1.2-fold. Contents obtained by quantitative immunoblots for GST subunits 1, 2 and 3+4 are also similar in both the homogenate and the nuclear fraction. The GST subunit 2 exhibits a 1.4-fold enrichment in the nuclear fraction.

Figure 1 compares the SDS/PAGE of the saline-soluble and -insoluble fractions from a typical nuclear preparation and the homogenate from which it was derived: all the GSTs are in the saline-soluble fraction, and none occurs as saline-insoluble chromatin protein. The histones are the predominant proteins in the nuclear saline-insoluble fraction (lane 5). The 1-chloro-2,4-dinitrobenzene-GST activity of the saline-soluble nuclear fraction is 1.6 $\mu\text{mol/min}$ per mg of protein. A decrease in GPx activity in the nuclear fraction compared with the homogenate, which is less than the decrease in the extranuclear markers NADPH-cytochrome *c* reductase or citrate synthase, indicates the presence of this enzyme in the nucleus, although with lower activity than in the extranuclear volume.

Table 1 Comparison of marker enzyme activities and contents of DNA, GSH and GSTs in homogenate and nuclear preparations obtained by non-aqueous fractionation of rat liver

Glutathione is given as GSH + GSSG, in GSH equivalents. All values refer to total (soluble + insoluble) protein of the respective fraction. Values are means $\pm$ S.E.M. ($n = 4-7$): *significantly different from homogenate. ^a From [34]; ^b from [38].

	Homogenate	Nuclei	Enrichment (fold)
DNA ($\mu\text{g}/\text{mg}$)	28.7 ± 2.7	$203 \pm 40^*$	7 9–10 ^a 10 ^b
Marker enzymes (m-units/mg)			
Nuclear			
NAD pyrophosphorylase	0.57 ± 0.03	$2.9 \pm 0.25^*$	5 7 ^a 9 ^b
Mitochondrial			
Citrate synthase	18.1 ± 1.2	$5.0 \pm 0.51^*$	0.28
Endoplasmic reticulum			
NADPH-cytochrome <i>c</i> reductase	25.0 ± 1.9	$7.8 \pm 0.7^*$	0.31
GST microsomal	2.3 ± 0.53	$0.89 \pm 0.22^*$	0.39
Total glutathione (nmol/mg)	19.0 ± 2.2	22.8 ± 5.8	1.2
GSTs ($\mu\text{g}/\text{mg}$)			
Total	32.0 ± 2.8	38.0 ± 5.0	1.3
GST-1	12.1 ± 1.3	14.4 ± 3.1	1.2
GST-2	6.9 ± 0.84	$9.8 \pm 0.9^*$	1.4
GST-3-4	11.5 ± 0.4	12.0 ± 1.4	1.0
GPx (m-units/mg)	86.0 ± 5.5	$56.0 \pm 7.0^*$	0.65

Figure 1 SDS/PAGE of saline-soluble and -insoluble fractions of a non-aqueous rat liver nuclear preparation and of the homogenate

Lanes 1 and 2 are from the homogenate and represent saline-soluble and saline-insoluble fractions, respectively. Lane 3 shows standard GSTs (G) and histones (H). Lanes 4 and 5 are saline-soluble and saline-insoluble nuclear fractions, respectively.

DISCUSSION

Nuclear fraction prepared by non-aqueous fractionation

It was recognized at an early time that separations in aqueous media might lead to the diffusion of water-soluble components from one cellular compartment to another, for instance between the nucleus and the soluble supernatant fraction [29,30]. Despite their obvious value, previously developed non-aqueous techniques have been used only rarely, probably because they are time-consuming, technically demanding and have required large quantities of starting material.

Fractionation procedures using heptane and CCl_4 as solvents were employed for the determination of mitochondrial and

cytosolic metabolite contents in liver [31] and heart tissue [32,33]. For the isolation of nuclei in non-aqueous solvents, previous studies used kidney [13,34]. This paper describes a modification of the non-aqueous fractionation procedure [14,34] as applied to the isolation of hepatocyte nuclei. It involves the sedimentation of a homogenate of freeze-clamped, freeze-dried perfused rat liver powder through H/C.

The use of organic solvents is based on the fact that metabolites and marker enzymes of interest are not extracted due to their insolubility in heptane and CCl_4 . This has been examined in previous work for polar metabolites such as citric acid-cycle metabolites and amino acids [35], and here also for glutathione, GSTs and GPx. Freeze-dried liver powder was sonicated as described in the Experimental section in H/C mixtures and centrifuged; less than 0.5% of GSH + GSSG content, GST and GPx activity were detected in extracts of the supernatant (results not shown). Fatty acyl derivatives with polar moieties are also not extracted [36]. Therefore, a redistribution of polar compounds during the non-polar fractionation can be excluded. Another point of interest is diffusion of metabolites or marker enzymes: due to lack of water, obviously there can be no diffusion of glutathione or GSTs in an aqueous phase. In the non-polar environment, cellular components such as marker enzymes and metabolites remain associated with their initial compartments.

Although the marker enzyme activities can be diminished slightly by the treatment in non-polar solvents, this does not discount their use as markers for a compartment. In studies using density gradient centrifugation in H/C for separation of mitochondria in liver, the sum of the activities of citrate synthase or NAD pyrophosphorylase in the fractions was similar to that of the homogenate, i.e. the recovery was complete [37]. Regarding GSTs, likewise the total activity in the homogenate was the same before and after sonication in H/C (results not shown).

Instead of the livers from 50–60 rats used in [34] and a procedure requiring 1 week, the present method uses only livers from two rats, and a period of 2 days is required. This method achieves a level of purity similar to that with the early method [34], and also a more recent method [38] in which anhydrous glycerol, metrizamide and 1,2-dichloropropane have been used to produce non-aqueous sedimentation media (Table 1). Using the present procedure, fractions may be obtained which have a higher enrichment in DNA than in NAD pyrophosphorylase activity. Slight segregation of DNA and NAD pyrophosphorylase is also apparent in the work reported in [34] and [38] (Table 1). This indicates that in broken nuclei a separation occurs of matrix containing DNA from matrix containing no DNA. Both markers, however, label the nuclear compartment. Therefore, compounds co-migrating with one of the nuclear markers can be assigned to the nuclear compartment. Sub-compartmentation of the nucleus is a possibility not addressed at this point.

Glutathione

Nuclear fractions obtained by the present method have a total glutathione content similar to that of the homogenate from which they were derived (Table 1). Thus the nucleus is rich in glutathione, and a concentration of 8.4 mM is calculated (Table 2), quite similar to the concentration in the cytosol, 8.2 mM, found after fractionation of liver using non-polar density gradient centrifugation for the study of mitochondrial and cytosolic compartments [31]. The previously reported loss of glutathione from nuclei isolated by aqueous techniques [3] is attributed to

Table 2 Subcellular GSH contents and concentrations obtained after fractionation of freeze-dried liver tissue in H/C

Contents are referred to compartmental protein. Nuclear concentrations have been calculated from contents, based on the following assumptions: (i) a cellular water space of 2.7 $\mu\text{L}/\text{mg}$ of protein [15]; (ii) a nuclear volume of 10% of cellular volume [28]; (iii) a nuclear protein of 10% of the cellular protein [34,38]. *Mitochondrial and cytosolic GSH contents and concentrations are from [31].

GSH	Nuclei	Mitochondria*	Cytosol*
Content (nmol/mg of protein)	22.8	8.8	31.0
Concentration (mM)	8.4	11.0	8.2

diffusion of glutathione through the nuclear membrane during isolation.

As mentioned above, in a study on the nuclear/cytoplasmic distribution of GSH, a nuclear/cytoplasmic gradient of 3 was detected in hepatocytes exposed to monochlorobimane, which, on diffusion into hepatocytes, yields a fluorescent monochlorobimane-GSH conjugate, dependent on GST catalysis [4]. It was suggested that monochlorobimane conjugations of nuclear and cytoplasmic GSH were independent of each other, and that the higher fluorescence observed in the nucleus, compared with the cytoplasm, was due to the presence of more GSH in the nucleus. Since the nuclear/cytoplasmic differential was found to be diminished when an uncoupler of mitochondrial oxidative phosphorylation, carbonyl cyanide *m*-chlorophenylhydrazone (CCCP), was present for a prolonged period, it was proposed that an ATP-dependent transport mechanism was involved in maintaining the gradient [4].

Briviba et al. [39] confirmed that exposure of hepatocytes to monochlorobimane extracellularly or by microinjection gave rise to the nuclear/cytoplasmic gradient in fluorescence as observed by [4]. However, they also showed that the same gradient was obtained after the microinjection of monochlorobimane-GSH or the conjugate with *N*-acetylcysteine, indicating that, in the work described in [4], the fluorescence was not necessarily due to intranuclear conjugation of nuclear GSH. Furthermore, other soluble fluorescent dyes, such as Lucifer Yellow and Cascade Blue, gave a similar distribution [39]. Thus it seems likely that the apparent GSH gradient described in [4] was due either to a longer light-path through the nucleus [40] or to a greater fluorescence yield in the nuclear environment. The observation of equilibration after prolonged exposure to CCCP is unexplained, but may be the result of cytotoxicity.

Glutathione derivatives transfer rapidly into the nucleus [39]. The glutathione distribution observed in the present work probably results from equilibration between glutathione in the free water phases of the nucleoplasm and the cytosol via the nuclear pores [41]. This also applies to the Ca^{2+} concentration in hepatocyte nuclei, as measured with specifically targeted recombinant aequorin [42]. Accumulation of Ca^{2+} inside the nucleus by a selective transport mechanism, as suggested in [43], was thought to be unlikely [42]. It is probable that the observed Ca^{2+} nuclear/cytoplasmic gradient, like those that have been observed for Na^+ and K^+ , is due to differential binding by the nucleus and cytoplasm [41].

GSTs

GST isoforms have a similar distribution between the nuclear fraction and the homogenate (Table 1). The SDS/PAGE analysis of the saline-soluble and saline-insoluble fractions of nuclei from

the non-aqueous separation shows that all detectable GST is saline-soluble (see also Figure 1), and no selective interaction between GSTs and chromatin as in nuclei isolated at low pH [11] is observed. In addition, the 1-chloro-2,4-dinitrobenzene-GST activity of the saline-soluble fraction was in the same range as the value calculated by using enzyme protein quantification shown in Table 1 and was similar to specific activities quoted in [2]. A specific activity of 0.7 unit/mg of protein was found previously in the cytosolic fraction of perfused rat liver, by using a similar non-aqueous fractionation technique (i.e. density-gradient centrifugation of freeze-dried liver powder in H/C [31]).

From Tables 1 and 2 it can be calculated that dimeric nuclear GSTs are present at a concentration of approx. 0.24 mM. Although Table 2 shows that the mitochondrion has similar levels of glutathione [31], its pattern of GSTs is quite distinct, due to the predominance of the mitochondrion-specific enzyme, GST 13-13 [44].

It is concluded that the absence of GSTs from the nuclear fraction, obtained by aqueous fractionations at neutral pH [9], is due to diffusion out of the nucleus during fractionation. Conversely, in aqueous fractionations at low pH [10,11], substantial changes in macromolecular charge may cause the accumulation of GSTs in the non-histone chromatin fraction.

From the present work it is apparent that, similarly to the cytoplasm, the nucleus is rich in freely diffusible glutathione and GSTs.

GPx

GPx activity has been demonstrated in all mammalian tissues. In rat liver, the selenoprotein is responsible for a substantial fraction of the activity [45]. The non-selenium-dependent activity is ascribed to GSTs [46]. Table 1 shows a selenium-dependent GPx activity lower in the nucleus than in the homogenate. Flohé et al. [47] did not find significant selenium-dependent GPx activity in nuclei isolated in sucrose media at neutral pH. However, as mentioned above, during aqueous separation at neutral pH enzyme protein might be lost. The activity has been determined with *t*-butyl hydroperoxide at 20 °C, and is therefore lower in the homogenate than values for liver homogenate determined at 37 °C [48].

From our data it is concluded that the nucleus possesses the complete GSH-dependent peroxidation protection system necessary to reduce hydroperoxides arising from oxidized nucleotides or DNA, i.e. glutathione, GST subunits 1, 2, 3 and 4, as well as selenium-dependent and selenium-independent GPx activities.

This work was supported by the Cancer Research Campaign, London, U.K., and by the National Foundation for Cancer Research, Bethesda, MD, U.S.A. B.K. was Forschungspreisträger of the Alexander-von-Humboldt-Stiftung, Bonn. We thank Bettina Schulz for help in initial measurements and Andrea Schlöhms and Harry Kramer for technical assistance.

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