EFFECTS OF CYSTEINE AND ACETAMINOPHEN ON THE SYNTHESES OF GLUTATHIONE AND ADENOSINE 3'-PHOSPHATE 5'-PHOSPHOSULFATE IN ISOLATED RAT HEPATOCYTES

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Abstract—The aim of the present study was to introduce and validate a radioactive tracer method in which adenosine 3'-phosphate 5'-phosphosulfate (PAPS) and glutathione (GSH) are measured simultaneously in isolated hepatocytes. PAPS and GSH are co-substrates in sulphation and GSH conjugation, and both are dependent on sulphur deriving from cysteine. The effect of cysteine on the syntheses was investigated at non-toxic and toxic concentrations of the hepatotoxic drug acetaminophen (AA). Administration of AA trapped radioactivity (^{35}S) in the pre-labelled PAPS and GSH pools by formation of the metabolites, AA-sulphate and AA-GSH. Turnover rates were determined from the decline of AA-sulphate and AA-GSH specific activity. Syntheses of PAPS and GSH were calculated by multiplying the rates with the concentrations of the respective co-substrates. Increasing AA concentration from non-toxic to toxic levels resulted in increased median PAPS and GSH syntheses (8 to 11 and 311 to 2218 nmol/10^6 cells/min, respectively) ($P < 0.05$). Addition of cysteine did not alter median PAPS synthesis (5 to 3 nmol/10^6 cells/min) but decreased median GSH synthesis (666 to 261 nmol/10^6 cells/ min) ($P < 0.05$) in experiments with *non-toxic* AA concentrations. In experiments with *toxic* AA concentrations opposite effects of cysteine were seen, i.e. median PAPS synthesis was reduced (3 to 2 nmol/10^6 cells/min) ($P < 0.05$) while median GSH synthesis was unchanged (23 to 16 nmol/10^6 cells/ min). The present method provides a tool in which two important detoxification pathways can be measured simultaneously and the data suggest that the two pathways are regulated by substrate availability.

Many xenobiotics are metabolized in the liver to form sulphate or glutathione conjugates. Sulphation is catalysed by cytosolic sulphotransferases (aryl sulphotransferase) (EC 2.8.2.1) and adenosine 3'-phosphate 5'-phosphosulfate (PAPS§) as co-substrate. Synthesis of PAPS requires ATP and inorganic sulphate [1] which predominantly is supplied by sulphur donors such as cysteine and methionine [2, 3]. Glutathione (GSH, γ-glutamyl-L-cysteinyl-glycine) is formed in the γ-glutamyl cycle in two steps. Glutamate and cysteine form α-glutamyl-cysteine by γ-glutamyl-cysteine synthetase (EC 6.3.2.2) and glycine is added by GSH synthetase (EC 6.3.2.3) to form reduced GSH. The intracellular GSH concentration is controlled by GSH negative feed-back on γ-glutamyl-cysteine synthetase [4]. GSH and inorganic sulphate share cysteine as a common precursor. It is, therefore, of interest to investigate GSH and PAPS syntheses simultaneously and the effect of cysteine administration.

The widely used analgesic acetaminophen (AA) produces liver necrosis in man and laboratory animals when ingested in large doses [5]. Most of the drug is sulphated and a minor fraction under-goes cytochrome P450 oxidation to form a reactive metabolite, N-acetyl-p-benzoquinoneimine (NAPQI) [6], which under normal circumstances is conjugated with GSH and excreted into urine or bile as N-acetyl-cysteine-acetaminophen (acetaminophen-mercapturate). After an AA overdose, excess amounts of NAPQI deplete hepatic GSH, and the reactive species covalently binds to hepatoproteins, which subsequently leads to cell death and tissue necrosis.

GSH synthesis has been measured *in vivo* in rats by the use of a radioactive tracer model [7]. The GSH pool prelabelled with [^{35}S]cysteine can be probed with AA. The turnover rate constant of GSH is calculated from the decline in the specific activity of the AA-mercapturate metabolite in bile.

In the present study, we have used the tracer model to measure PAPS and GSH syntheses simultaneously in rat hepatocytes, and studied the common regulation of the co-factors of these two important detoxification pathways.

MATERIALS AND METHODS

Female Wistar rats (180–205 g, Møllegårdens Avlslaboratorium, Ll. Skensved, Denmark) were fasted for 16 hr before the experiments. The animals were kept in an air-conditioned environment with a controlled 12 hr light–dark cycle. They had free access to water. One hour before hepatocyte isolation, the rats were given [^{35}S]L-cysteine

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§ Abbreviations: PAPS, adenosine 3'-phosphate 5'-phosphosulfate; GSH, glutathione; AA, acetaminophen; NAPQI, N-acetyl-p-benzoquinoneimine.

$(80\text{--}130\,\mu\text{Ci}$, sp. act. $>600\,\text{Ci/mmol}$, Amersham Denmark ApS) intraperitoneally.

Hepatocyte isolation from rats was performed after collagenase perfusion [8, 9]. The yield of each preparation was $0.1\text{--}8.0 \times 10^8$ cells per liver, measured by counting the final cell suspension in a Buerker chamber. Immediately after isolation, 80–91% of the cells excluded Trypan blue. Incubation was performed at 37° under a 95% O_2/5% CO_2 atmosphere at a cell concentration of 10×10^6 cells/mL. The incubation medium was a Krebs–Ringer buffer ($MgSO_2$ 1.2 mM, pH 7.4) supplemented with glucose (10 mM) and albumin (1% w/v). AA (0.2 and 5.0 mM) was added to shaken incubation flasks immediately before starting the experiments. In other experiments, AA (0.1 or 5.0 mM) was added to the incubation flasks together with L-cysteine (0 and 6.8 mM) immediately before starting the experiment. AA served a dual purpose: (1) trapping of the sulphur coming from the co-substrates PAPS and GSH, and (2) perturbing the cysteine pool. At low AA concentration the influence on the cysteine pool is negligible [7], whereas at high AA concentration the cysteine pool is depleted.

Analytical methods. PAPS in isolated hepatocytes was analysed using a modification of a method of Hazelton *et al.* [10]. The assay measures the formation of α-[^{14}C]naphthyl sulphate from α-[^{14}C]-naphthol and PAPS (limiting substrate) via a sulphotransferase-catalysed reaction. One millilitre of incubate was immediately boiled in 2 vol. of 50 mM glycine/NaOH buffer (pH 9.2) for 3 min. The boiled tissue was cooled on ice and stored at −80° until the following day. The homogenate was centrifuged at 20,000 g for 20 min and the resultant supernatant was decanted and extracted with 3 mL chloroform by vortexing for 1 min. The aqueous and organic phases were separated by centrifugation at 20,000 g for 20 min, and the aqueous phase used immediately for the analysis of PAPS. Three hundred microlitres of tissue extract or PAPS standard was incubated in the assay containing 200 mM Tris–HCl (pH 7.4), 2.5 mM $KH_2PO_4 \cdot K_2HPO_4$, 5.0 mM EDTA, 0.75 mg cytosolic protein with standardized sulphotransferase activity and 0.25 mM α-[^{14}C]-naphthol (0.81 μCi/μmol). Cytosolic sulphotransferase was prepared according to the procedure described by Hazelton *et al.* [10]. Inter-batch standardization was based on individual and freshly prepared PAPS standard curves. The reaction mixture was incubated at 37° in a shaking water bath for 60 min. After incubation, the reaction was terminated by addition of 500 μL ethanol and acidification with 100 μL 2 M sodium acetate (pH 4.8). Conjugated and unconjugated naphthol were extracted for 60 min with 3 mL chloroform and samples were centrifuged at 2500 g for 10 min. Radioactivity was determined in a 300 μL aliquot of the aqueous phase by liquid scintillation spectrometry (Packard Instruments), with quench correction by the channels ratio technique and a precision of 1% (2 sigma). Standard curves were linear for 2–15 mM PAPS, with recovery at 80–110%. The overall variability in the PAPS assay was 4% (1–7) (coefficient of variation) including all experiments (N = 19). The assay included measurement of the

^{35}S-label from cysteine. This fraction depended linearly on the amount of [^{35}S]cysteine used in the experiments and the dependency was used to correct for overestimation of the PAPS pool.

Total glutathione in the cell incubate was measured by the glutathione reductase 5,5′-dithiobis (2-nitrobenzoic acid) assay of Tietze [11]. One hundred microlitres of the incubate were added to 100 μL perchloric acid (1 N), and after centrifugation the supernatant was stored at −20° until analysis. GSH is stable for 3 weeks by this procedure. The variability of the GSH assay did not differ from previous studies in this laboratory [12] with a variability of 7% (coefficient of variation).

For determination of the specific activity of the AA-sulphate and the AA-GSH metabolites formed by isolated hepatocytes, 250 μL of the incubate was precipitated with 50 μL 4 N perchloric acid, and after centrifugation 200 μL of the supernatant was injected into the HPLC (Waters Associates). The metabolites were separated on a C_{18}-μBondapak column (Waters Associates) with water:methanol:acetic acid (91:8:1, by vol.), KH_2PO_4 0.05 M, 1.5 mL/min as mobile phase [13]. The identity of the AA-sulphate metabolite eluting at 5 min was established using synthetic standard (Sterling-Winthrop). The identity of the AA-GSH metabolite eluting at 12 min was confirmed by comparison of the HPLC retention time of the metabolite found by Moldéus [14] in isolated hepatocytes. Quantification of the metabolites was accompanied using AA as a standard since the molar extinction coefficients are essentially the same [15]. Radioactivity in the collected AA-sulphate and AA-GSH peaks was determined by liquid scintillation spectrometry with quench correction by the channels ratio technique and a precision of 1% (2 sigma).

Calculations. Development and validation of an *in vivo* method which assessed hepatic GSH turnover by acetaminophen probe analysis was introduced by Lauterburg in 1980 [7]. Lauterburg probed a pre-labelled GSH pool by AA and collected the AA-GSH adduct in bile at various time intervals or time windows for specific activity determination. GSH turnover rate was determined from the mono-exponentially decline of the adducts in bile:

$$\text{sp. act.} = \exp\left(-k \times t\right).$$

In our experimental design, the radioactive metabolites are present in the incubation media throughout the experiment, and therefore we were not able to use a simple first order monoexponentially decay. However, the differential equation

$$\text{sp. act.} = \frac{1}{t}\int_0^t \exp\left(-k \times t\right)\mathrm{d}t$$

meets these demands. In order to increase the degrees of freedom and to explore that the experimental data were paired with identical initial specific activity, a constant B was implemented in the equation. The regression line approached exponentially a lower limit A which also was

implemented in the equation. Finally the turnover rates were calculated according to

$$\text{sp. act.} = \frac{B}{t}\int_0^t \exp(-k \times t)\mathrm{d}t + A$$

which gave

$$\text{sp. act.} = \frac{B}{k \times t}[1 - \exp(-k \times t)] + A.$$

k was found by non-linear least squares iterative regression analysis by the use of BMDP statistical software (BMDP, UCLA, 1985). Calculations of paired turnover rate contants, i.e. two matching data sets, were performed simultaneously by the use of indicator variables I_1 and I_2 (given values 1 and 0, respectively) holding B constant. This gave

$$\text{sp. act.} = I_1 \frac{B}{k_1 \times t}[1 - \exp(-k_1 \times t)] + A_1$$

$$+ I_2 \frac{B}{k_2 \times t}[1 - \exp(-k_2 \times t)] + A_2.$$

Syntheses of PAPS and GSH were calculated by multiplying the rate constants k_{PAPS} and k_{GSH} with the mean concentration of their respective co-enzymes from 0 to 30 min ($PAPS_{0-30min}$ and $GSH_{0-30min}$, respectively).

All values are expressed as median with interquartile distances. The differences between turnover rates, concentrations and syntheses are tested using a Wilcoxon test for paired observations. The level of significance was 0.05.

RESULTS

The fitting procedure resulted in a variability of the PAPS and GSH turnover rates of 31% (11–84) and 20% (10–88), respectively (N = 38) (coefficients of variation). The decline of the specific activity of the AA-sulphate and AA-GSH metabolites is shown in Fig. 1 (lower left and right) in a representative experiment in which hepatocytes were incubated with non-toxic (0.2 mM) and toxic (5.0 mM) AA concentrations. In addition, Fig. 1 (upper left and right) shows PAPS and GSH concentrations in the same experiment.

Table 1 shows turnover rate constants, mean chemical concentrations from 0 to 30 min and syntheses of PAPS, GSH, AA-sulphate and AA-GSH in paired experiments with hepatocytes incubated with 5.0 and 0.2 mM AA (experiment I), 0.1 mM AA and 6.8 mM cysteine (experiment II) and 5.0 mM AA and 6.8 mM cysteine (experiment III).

In experiment I PAPS and GSH syntheses were increased by 51% and 713%, respectively (P < 0.05). In contrast, the high AA concentration did not influence PAPS or GSH concentrations. AA-sulphate and AA-GSH syntheses were increased by 87% and 431%, respectively (P < 0.05). Figure 1 shows data from a representative experiment, together with the fitted specific activity curves. Figure 2 shows syntheses of PAPS and GSH (per

cent of syntheses at 0.2 mM AA) at high (5.0 mM) and low (0.2 mM) AA levels.

In experiment II cysteine did not influence PAPS or AA-sulphate syntheses at low, non-toxic AA levels, even though the PAPS concentration was raised slightly by 8% after cysteine supplementation (P < 0.05). In contrast, both GSH and AA-GSH syntheses were reduced by 61% and 25%, respectively (P < 0.05) when cysteine was present in the media. The GSH concentration was, however, raised by 19% (P < 0.05). Figure 3 shows syntheses of PAPS and GSH after cysteine addition (per cent of control) at low AA levels.

In experiment III opposite effects were seen compared to experiments with 0.1 mM AA. Even though the concentration of PAPS was raised by 6% (P < 0.05), PAPS synthesis was reduced by 43% (P < 0.05) without affecting AA-sulphate synthesis. GSH synthesis did not change even though GSH concentration was raised by 7% (P < 0.05) and AA-GSH synthesis reduced by 20% (P < 0.05). Figure 4 shows syntheses of PAPS and GSH after cysteine addition (per cent of control) at high AA levels.

DISCUSSION

Perturbation of cysteine, essential for the formation of the important detoxification co-substrates GSH and activated sulphate (PAPS), by high AA concentration increased the immediate synthesis of both PAPS and GSH dramatically. When the cysteine pool is unperturbed, we found a synthesis of PAPS of about $8 \text{ nmol}/10^6 \text{ cells/min}$, and a synthesis of GSH of about $311 \text{ nmol}/10^6 \text{ cells/min}$. High concentration of cysteine did not affect PAPS synthesis, but reduced GSH synthesis by two-thirds in experiments with low AA concentration. The opposite effect of cysteine was seen in experiments with high AA concentration. PAPS synthesis almost halved while GSH synthesis was unaffected. These data suggest a common regulation of the co-substrates for sulfotransferase and GSH-S-transferase and sensitivity to changes in the concentration of their common precursor cysteine.

An in vivo study by Lauterburg et al. [7] who probed a radioactively labelled GSH pool with AA showed that rats fasted for 48 hr had a fractional rate of GSH turnover of 0.0067 min^{-1}. This is in contrast to a GSH turnover rate of about 25 min^{-1} in our study of isolated hepatocytes. However, Lauterburg et al. measured GSH turnover 1–3 hr after AA was administered, while we measured GSH turnover from 0 to 30 min, the time period in which the decline of specific activity in the AA metabolites is most rapid. The existence of two intracellular GSH pools, a rapid exchangeable and a slow, intramitochondrial pool has been debated [16]. Consequently, the substantial difference of GSH turnover rate constants between the two studies could partly be explained by the fact that we measure only the first and Lauterberg et al. only the latter GSH pool. Another factor responsible for the increased GSH turnover might be the fact that freshly isolated hepatocytes are under enormous oxidative stress [17, 18] with increased GSH peroxidase activity and increased GSH consumption

PAPS
concentration

GSH
concentration

Specific activity
AA-sulphate

Specific activity
AA-GSH

min

min

● AA   ○ AA
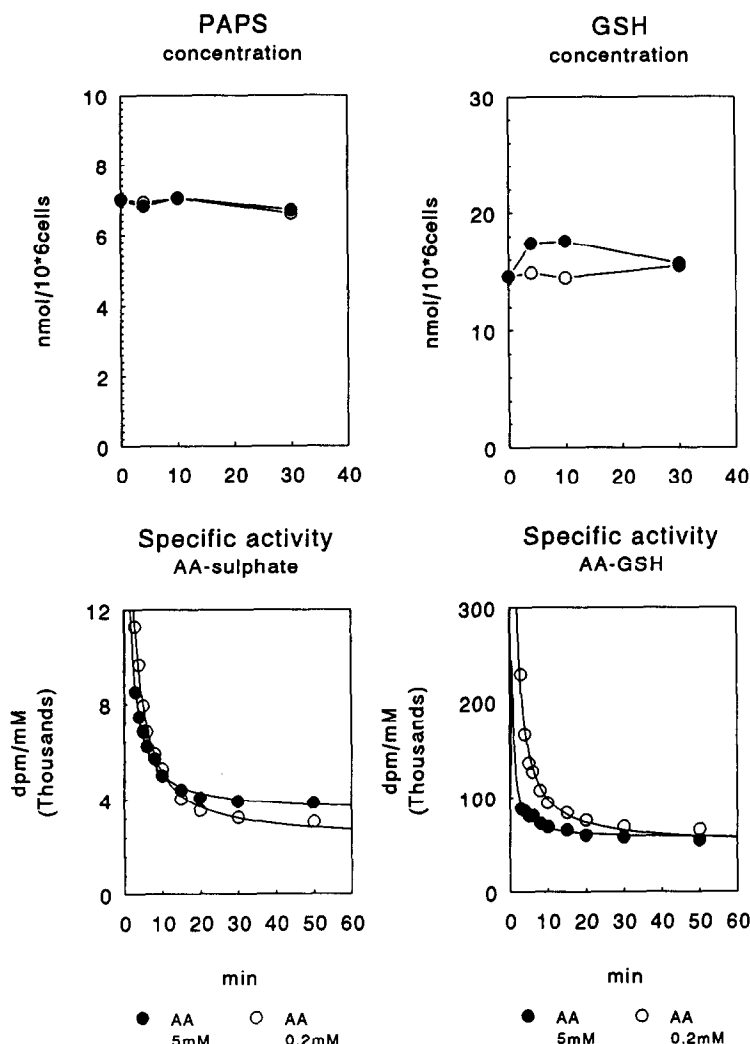 5mM   0.2mM

● AA   ○ AA
  5mM   0.2mM

Fig. 1. PAPS concentration (upper left), GSH concentration (upper right), AA-sulphate specific activity (lower left) and AA-GSH specific activity (lower right) in a representative experiment in which rat hepatocytes were incubated with 5.0 mM (filled circles) and 0.1 mM AA (open circles). Specific activity curves (lower left and right) were fit to the expression

$$\text{sp. act.} = \frac{B}{k \times t}[1\text{-exp}(-k \times t)] + A$$

where B is the initial specific activity, k is the turnover rate constant and A is the lower limit of which the regression line approaches exponentially.

leading to decreased intracellular concentration and subsequently to increased GSH turnover. In fact the concentration found in our study is lower than other studies [18, 19].

The slow decline of AA-GSH specific activity seen after 30 min might be tracer "washout" from the deep GSH pool or it might be tracer derived from protein breakdown. Although incorporation and breakdown of tracer from proteins is slow, this is a possibility since 3–4 hr passed between [35S]cysteine administration and AA treatment of the isolated cells. The suppressed GSH synthesis seen *in vivo* in fasted and fed rats after AA (1 g/kg) [20] could not

be found at toxic 5.0 mM AA concentration in the present study. However, we estimated k_{GSH} in the early interval 0–30 min in which GSH probably is not depleted enough resulting in a k_{GSH} significantly increased compared to the one found after 0.1 mM AA. Lauterberg *et al.* [7] calculated a fractional turnover rate 1–3 hr after AA administration, i.e. subsequent to GSH depletion. In our system GSH is depleted to about 50% of initial values (AA 5.0 or 10.0 mM) after 2 hr (data not given), also indicating that measurements relate to a situation where GSH is not completely depleted.

Increased syntheses of both PAPS and GSH after

Table 1. Median (interquartile distances) turnover rates, concentrations, syntheses of PAPS, GSH, AA-sulphate and AA-GSH in isolated hepatocytes incubated with 0.2 and 5.0 mM AA (experiment I) (N = 6), 0.1 mM AA and 6.8 mM cysteine (experiment II) (N = 7) and 5.0 mM AA and 6.8 mM cysteine (experiment III) (N = 6)

Experiment	Turnover rate of PAPS (min)		Concentration of PAPS (nmol/10^6 cells)		Synthesis of PAPS (nmol/10^6 cells/min)		Synthesis of the AA-sulphate adduct (nmol/ 10^6 cells/hr)	
I Low AA	1.1	(0.2–9.8)	1.94	(0.34–6.81)	7.45	(0.08–14.95)	11.6	(9.0–13.8)
High AA	2.5	(1.5–19.0)	2.02	(0.44–6.87)	11.23*	(0.21–29.94)	21.5*	(13.7–24.7)
II Low AA	0.7	(0.3–14.3)	2.28	(1.88–2.47)	4.86	(0.26–30.25)	5.1	(4.7–5.5)
+ Cysteine	0.5	(0.3–13.8)	2.47†	(2.02–2.81)	3.10	(0.56–27.88)	5.2	(4.8–5.4)
III High AA	1.3	(1.0–2.3)	2.18	(1.97–2.48)	2.86	(2.04–5.21)	20.3	(19.1–26.8)
+ Cysteine	0.7†	(0.4–1.4)	2.28†	(2.33–2.68)	1.62†	(0.89–3.20)	20.0	(19.1–26.5)

Experiment	Turnover rate of GSH (min)		Concentration of GSH (nmol/10^6 cells)		Synthesis of GSH (nmol/10^6 cells/min)		Synthesis of the AA-glutathione adduct (nmol/ 10^6 cells/hr)	
I Low AA	16.2	(2.4–31.3)	16.2	(14.9–21.7)	311	(37–516)	1.0	(0.7–1.2)
High AA	106.6*	(32.3–150.0)	17.5	(16.1–21.3)	2218*	(520–3193)	5.4*	(3.3–5.5)
II Low AA	32.5	(20.8–40.3)	18.8	(17.2–20.6)	666	(234–830)	0.4	(0.4–0.5)
+ Cysteine	15.3†	(4.4–28.5)	22.3†	(18.7–25.0)	261†	(82–616)	0.3†	(0.2–0.3)
III High AA	1.7	(0.5–4.2)	17.4	(13.2–19.5)	23	(10–81)	5.0	(3.6–5.6)
+ Cysteine	1.2	(0.2–2.7)	18.6†	(13.9–20.7)	16	(3–56)	4.0†	(2.6–4.5)

* Significant different (P < 0.05) from paired experiments with low AA.
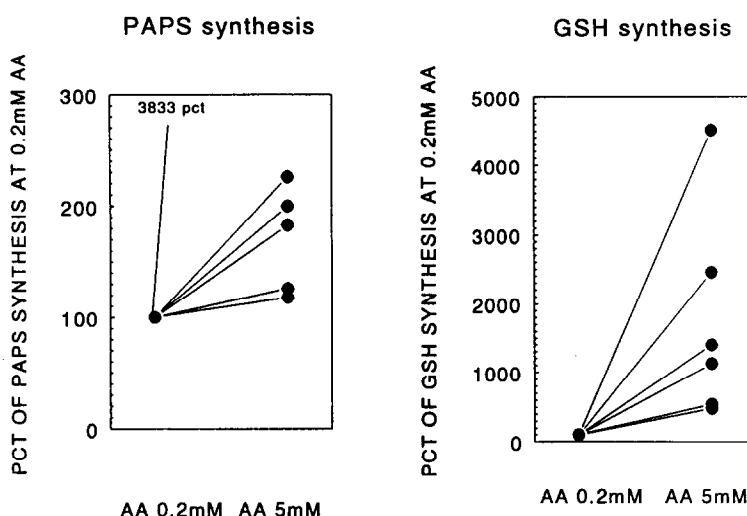† Significant different (P < 0.05) from paired experiments without cysteine.

Fig. 2. PAPS and GSH syntheses in experiments in which rat hepatocytes were incubated with 0.2 and 5.0 mM acetaminophen.

increased substrate concentration are consistent with overall sulphation and GSH conjugation following Michaelis–Menten kinetics. Determination of the enzymatic constants V_{max} and K_m for PAPS and GSH syntheses has not been performed.

Cysteine is capable of reducing the reactive AA metabolite, NAPQI, back to AA, thus forming less substrate for GSH conjugation [21] leading to decreased GSH synthesis. Indeed, this might be the explanation for the results seen at 0.1 mM AA levels. In contrast, the substrate for GSH conjugation (AA) is still present in a very high concentration, not resulting in reduced synthesis, in experiments with high AA levels. Lauterburg et al. [21] found increased GSH synthesis after cysteine administration, but in contrast to our study his GSH pool was depleted.

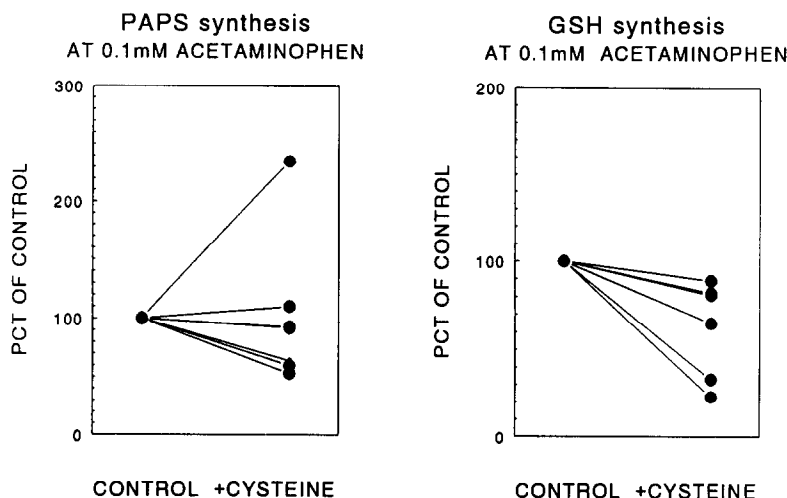Both PAPS and AA-sulphate syntheses are increased after administration of a toxic AA dose.

PAPS synthesis
AT 0.1mM ACETAMINOPHEN

GSH synthesis
AT 0.1mM ACETAMINOPHEN

Fig. 3. PAPS and GSH syntheses in experiments in which rat hepatocytes were incubated with 0.1 mM
AA without or with 6.8 mM cysteine.

PAPS synthesis
AT 5mM ACETAMINOPHEN
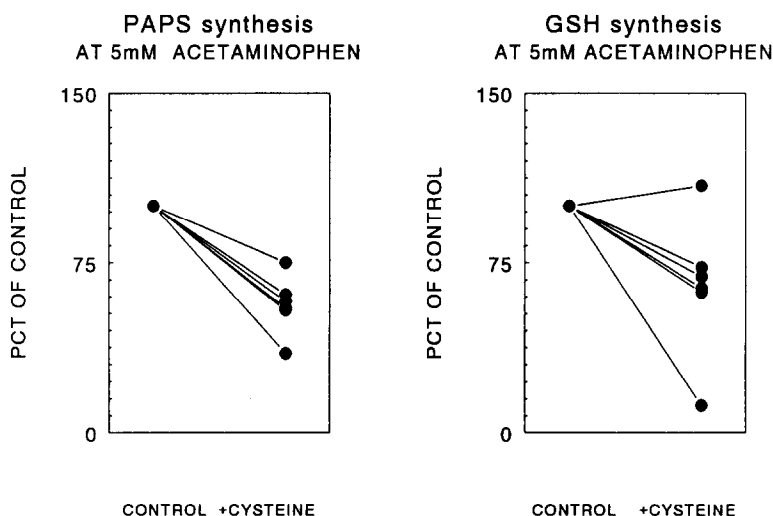
GSH synthesis
AT 5mM ACETAMINOPHEN

Fig. 4. PAPS and GSH syntheses in experiments in which rat hepatocytes were incubated with 5.0 mM
AA without or with 6.8 mM cysteine.

However, when cysteine as an organic sulphur donor is given to the hepatocytes incubated with 5.0 mM AA, PAPS synthesis is significantly reduced without affecting AA-sulphate synthesis. Sulphate adenylyltransferase (ATP-sulfyrulase) (EC 2.7.7.4), one of two PAPS synthesizing enzymes, is strongly inhibited by its products [22]. When cysteine is given, the concentrations of the PAPS intermediates adenosine 5'-sulphatophosphate and pyrophosphate are increased and PAPS synthesis consequently reduced. This did not affect AA-sulphate formation, indicating that sulphotransferase activity was rate-limiting in sulphation of toxic AA doses. At low, non-toxic AA concentrations, cysteine supplementation did not affect PAPS synthesis, indicating that

PAPS intermediates were present in non-inhibitive concentrations and that there was no draw of sulphur from the cysteine pool. Recycling of AA-sulphate has not been demonstrated, but is a possibility that must be considered, due to the unproportional increase in PAPS synthesis compared to the formation of AA-sulphate.

An assumption for the present method is that the concentrations of PAPS and GSH are constant during incubation. As can be seen from Fig. 1 (upper right), this was not the case in all experiments. Generally, the GSH pool increased after cysteine administration at low AA concentration, and was beginning to decrease at high AA concentrations without cysteine present. Therefore we decided to

use mean concentration from 0 to 30 min after incubation, i.e. the time period in which the specific activity of the AA metabolites decreased most rapidly. One could speculate that the decreased GSH turnover rate, seen in experiments with low AA concentration, was the result of increased GSH pool size. However, if a mean GSH concentration from *0 to 10 min* was used for the calculation of GSH synthesis, the result would still be the same as with a mean GSH concentration of *0 to 30 min*. Addition of "cold" cysteine would speed up disappearance of tracer from the GSH pool. However, turnover rates are still reduced which indicates that the rates would be even lower, if the GSH pools were identical with or without cysteine. The same argument goes for the reduction of PAPS turnover rates seen in experiments with high AA concentration, although differences in PAPS pools with or without cysteine were minimal.

The large variability between the various experiments can partly be explained by the method by which we establish cell viability and cell concentration i.e. exclusion of Trypan blue. Enzyme leakage or ATP concentration are alternative ways of measuring cell viability/concentration and variation between the different methods cannot be excluded. However, exclusion of Trypan blue is an easy applicable method and well-recognized in *in vitro* studies with isolated hepatocytes. The large difference between our experiments is most noticeable in the GSH data (turnover rates and syntheses) compared with the PAPS data. If this was only due to methodology we would expect the inter-experimental variation to be within the same magnitude. In humans there is a well-defined genetic polymorphism in the expression of the mu-class GSH-*S*-transferase gene cluster [23, 24] resulting in different ability to detoxificate xenobiotics or carcinogens. This polymorphism might indeed play an important role in the large inter-experimental variation.

In summary, this study introduces a method which simultaneously measures the synthesis of GSH and PAPS, together with syntheses of their respective AA metabolites in a simple *in vitro* system, hereby providing simultaneous information about two important detoxification pathways, i.e. sulphation and GSH conjugation. Both PAPS and GSH syntheses were stimulated in isolated hepatocytes by increasing the AA concentration from 0.2 to 5.0 mM in the incubation medium. Addition of cysteine, a sulphur donor for GSH and PAPS syntheses, decreased GSH synthesis at *low AA doses*, leaving PAPS synthesis unaffected. At *high AA doses* opposite effects were seen. The present radioactive tracer model provides a valuable tool in which the regulation of GSH and PAPS metabolism can be studied. Methods to increase syntheses of both compounds, hereby increase detoxification of xenobiotics, might also be found using the present approach.

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