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# GASTROINTESTINAL METABOLISM OF N-ACETYLCYSTEINE IN THE RAT, INCLUDING AN ASSAY FOR SULFITE IN BIOLOGICAL SYSTEMS

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## ABSTRACT

The intestinal metabolism of N-acetylcysteine was studied in the rat. Isolated intestinal epithelial cells were shown to rapdily deacetylate [$^{14}$C]-N-acetylcysteine to [$^{14}$C]-cysteine, with slight oxidation of the latter to disulfide species. The cells did not accumulate reduced or oxidized cysteine, and N-acetylcysteine itself was not detected either free or in oxidized species intracellularly. Further metabolism of this NAC-derived cysteine to inorganic sulfite or glutathione was not detected. Following the administration of [$^{14}$C]-N-acetylcysteine (50 mg/kg; 25 μCi) *in vivo* into the ilium, small quantities of both reduced and oxidized [$^{14}$C]-N-acetylcysteine were demonstrated in hepatic portal vein plasma. [$^{14}$C]-cysteine and inorganic sulfite were demonstrated as the major metabolites of N-acetylcysteine. These were present in the portal vein plasma at levels five and three times greater than the parent drug, respectively, 30 min after dosing. Additionally, [$^{14}$C]-glutathione was shown to be a minor metabolite of N-acetylcysteine accumulating in portal vein plasma.

These results may provide an explanation for the apparent low bioavailability of N-acetylcysteine when administered orally in humans and are discussed in terms of the origins of the protective effect of the drug in cases of paracetamol intoxication in humans.

KEY WORDS N-acetylcysteine Gut metabolism Sulfite assay

## INTRODUCTION

The therapeutic importance of N-acetylcysteine (NAC) is now well established. Despite this, there has been little information concerning the disposition and metabolic fate of the drug in animals or man.

Recently we have described a series of analytical techniques for the analysis of free, reduced NAC as well as oxidized NAC involved in disulfide species in most biological sysems.[1] These techniques also allow the simultaneous

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analysis (with NAC) of endogenous thiol components such as cysteine (cys), glutathione (GSH) and protein thiols.[2]

We have initially applied these techniques to a study of the plasma pharmacokinetics and urinary excretion of NAC following single oral and intravenous administrations of the drug to a volunteer.[1] The results strongly indicated that metabolism plays a pivotal role in the clearance of this drug when given by either route, and that when given orally, as is most common, NAC probably undergoes extensive first-pass metabolism.

Thus, in order to delineate possible biotransformations contributing to the metabolism of orally administered NAC, we report here the use of the above analytical techniques, extended to include the assay of inorganic sulfite, in studies of the intestinal metabolism of NAC in the rat.

## MATERIALS

[$^{14}$C]-labelled NAC and all materials for the analysis of thiols in biological systems were obtained as described previously.[1,2] Anhydrous sodium sulfite (>99 per cent) was obtained from Sigma Chemicals, St Louis, U.S.A. All materials for the isolation of rat intestinal epithelial cells were as previously stated.[3] Male Sprague–Dawley rats (190–210 g) were used throughout the experiments. Animals were allowed food and water *ad libitum*.

## METHODS

### *Isolation and incubation of intestinal epithelial cells*

The isolation of intestinal epithelial cells from rats was performed essentially by the protease digestion technique of Dawson and Bridges,[3] however isolated cells were washed additionally once with Krebs buffer pH 7·4 containing Hepes (25 mM) before use. Cells were incubated at a concentration of $5 \times 10^6$ cells/ml in fresh Krebs at 37° C in sililized flasks under an air atmosphere. In some experiments [$^{14}$C]-NAC (2 μCi) was included in the incubations. Samples of total incubation (cells plus medium), medium only and cells only were assayed for reduced and oxidized thiol components as described previously.[1,2] Where necessary samples of h.p.l.c. eluent were collected and counted for $^{14}$C by liquid scintillation spectrometry (Betarak, LKB, Bromma, Sweden).

### *Pharmacokinetic experiments*

Animals were anaesthetized with pentabarbital (60 mg/kg i.p.) prior to surgery. The abdomen was opened with a mid-line incision, and the hepatic portal vein cannulated (PE 20) distal to the liver, leaving as large a portion of the mesentaric drainage as possible undisturbed. A segment (5 cm) of the ilial section of the small intestine was ligatured-off from the remainder of the gut.

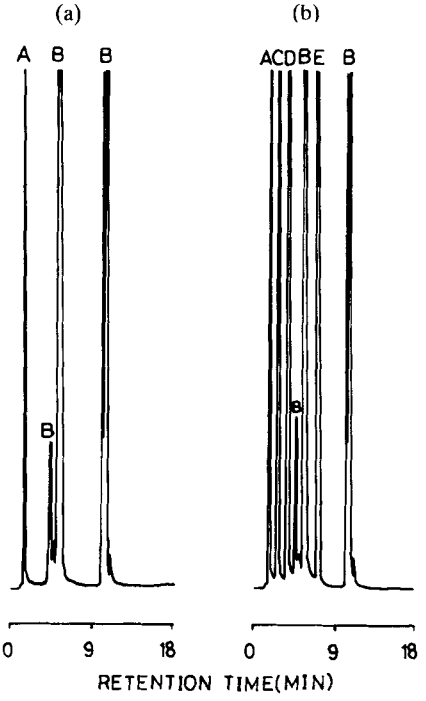


Figure 1. Typical h.p.l.c. traces from the analysis of sulfite in biological systems with mBBr: (a) $SO_3^{2-}$ (100 μM) in Krebs–Hepes pH 7·4 buffer derivatized with mBBr; (b) as (a) with the addition of 100 μM each cysteine, GSH and NAC. Peak A = $SO_3^{2-}$–mBBr adduct peak, r.t. 1·7 min; peak B = reagent peaks; peak C = cysteine–mBBr adduct peak, r.t. 2·4 min; peak D = GSH–mBBr adduct peak, r.t. 3·7 min; peak E = NAC–mBBr adduct peak, r.t. 7·0 min. Integrator attenuation $2^5$ mV

Blood samples (0·5 ml) were obtained immediately prior to, and at regular intervals after, a dose of NAC (50 mg/kg, 25 μCi $^{14}C$) in 0·5 per cent $NaHCO_3$ (1 ml) was inserted into the isolated intestinal segment. Blood was collected into heparinized tubes and plasma separated by centrifugation at 1500 × g for 5 min at 4° C. Plasma was analysed for reduced and oxidized thiols as above.

*Assay of sulfite in biological systems*

Inorganic sulfite ($SO_3^{2-}$) was analysed in samples of cellular incubations and blood plasma following derivatization of samples with monobromobimane (mBBr) and h.p.l.c. with fluorescence detection as described previously.[1,2]

It may be seen from Figure 1(a) that mBBr reacts with inorganic sulfite in basic solution to yield a fluorescent adduct, which is well separated (r.t. 1·6 min) from both reagent hydrolysis peaks and thiol-mBBr adduct peaks (Figure 1(b)) in the h.p.l.c. system used.

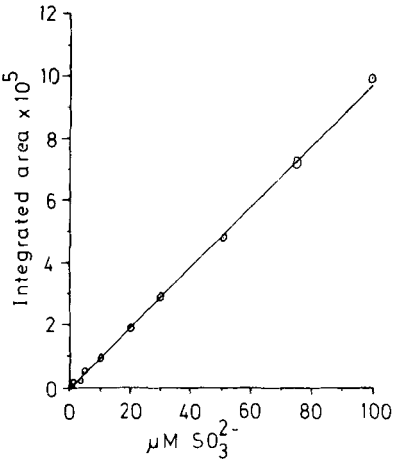


Figure 2. A typical calibration curve from the analysis of sulfite in biological systems with mBBr. Line equation is $y = 9{\cdot}8\,x$; $r^2 > 0{\cdot}997$

Calibration curves, typically constructed over the concentration range 1 to 100 μM $SO_3^{2-}$ (Figure 2), gave linear instrument response over the full range ($r^2 \geqslant 0{\cdot}997$), passing through the origin. Under the conditions of assay, the recovery of $SO_3^{2-}$ as its mBBr derivative was maximal at all times (assessed by the use of $^{35}SO_3^{2-}$) and unaffected by spiking $SO_3^{2-}$ into plasma. Calibration curves were unaffected by $SO_3^{2-}$ concentrations up to 1 mM and the presence of low molecular weight thiols up to a total concentration of 2 mM. Variability of calibration points, absolute limit of detection and assay sensitivity and precision for $SO_3^{2-}$ are similar to the corresponding values reported for the assay of NAC.[1]

## RESULTS

### *The metabolism of NAC by isolated intestinal epithelial cells*

Figure 3 shows results of the analysis of free and total cysteine and NAC in samples of cells plus medium from control cellular incubations and those supplemented with NAC (100 μM).

Immediately following addition of cells, control incubations contained small amounts of free cysteine and GSH (2·0 and 20·0 nmol/ml respectively), with only small amounts of oxidized cysteine present, bringing the total cysteine content up to 6·0 nmol/ml incubation. These control free and total cysteine levels were shown to increase throughout the incubation period, giving levels of 8·0 and 24·0 nmol/ml incubation, respectively, after 60 min. Conversely, control-free GSH levels were shown to steadily decrease throughout the time period to 10·0 nmol/ml at 60 min (data not shown).

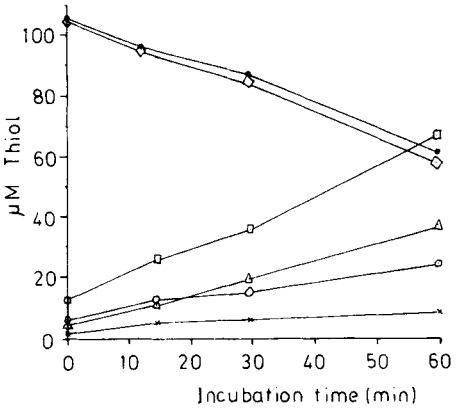


Figure 3. The metabolism of NAC to cysteine by isolated rat intestinal epithelial cells. Controls; ×——× free cysteine; ○——○ total cysteine following reduction of sample with DTT. + NAC (100 μM); △——△ free cysteine; □——□ total cysteine following reduction of sample with DTT; ◊——◊ free NAC; ●——● total NAC following reduction of sample with DTT. Data are expressed as means of three experiments, with an s.e.m. < ±6 per cent on all points. Samples were of complete incubation

When NAC (100 μM) was included in the incubations, the concentration of the free drug fell by *ca.* 50 per cent in 60 min. This was shown not to be due to chemical oxidation of NAC to disulfides. Concurrently, there were greater-than-control increases in both free and total cysteine, resulting in levels of 37 and 67 nmol/ml incubation, respectively, after 60 min. In contrast, free GSH levels were unaltered with respect to controls throughout the incubation.

These results were reflected almost exactly when the extracellular medium was assayed alone at each time point, except for the absence of free GSH.

Assay of the cells only demonstrated that free and total cysteine did not accumulate intracellularly during the incubation and failed to demonstrate free or disulfide-bound NAC intracellularly at any time point. Inorganic sulfite was not detected in the incubations at any time.

From the inclusion of [$^{14}$C]-NAC in the incubation, radioactivity was demonstrated associated with the NAC–mBBr adduct peak at the beginning of the incubation (Figure 4(a)) and in both the NAC–mBBr (although reduced) and cys–mBBr peaks following 60 min of incubation (Figure 4(b)).

*The intestinal metabolism of NAC in vivo*

The results of analysis of rat hepatic portal vein plasma levels of free and total sulfite, cysteine, GSH and NAC following intraintestinal administration of NAC are shown in Figure 5. Immediately prior to NAC administration, portal vein plasma was shown to contain levels of free sulfite, cysteine and GSH of 2·8, 2·0 and 0·8 nmol/100 μl plasma, respectively (Figure 5(A)). Reduction of the plasma prior to derivatization yielded total concentrations

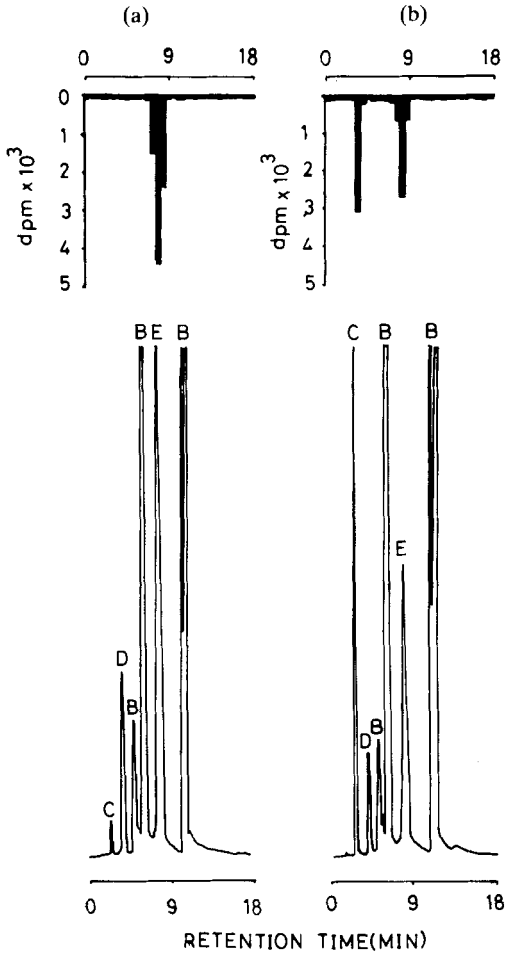


Figure 4. Radiochromatographic traces obtained from epithelial cell incubations: (a) immediately after addition of [$^{14}$C]-NAC and (b) 60 min after this. Peak assignment and integrator attenuation as in Figure 1

of these components of 6·0, 14·5 and 2·0 nmol/100 µl plasma, respectively (Figure 5(B)).

Following dosing, free NAC was shown to steadily accumulate in portal vein plasma up to a level of 3·9 nmol/100 µl after 30 min. Concurrent to this, there were significant increases in plasma free sulfite, cysteine and GSH, giving levels of 12·9, 19·8 and 2·7 nmol/100 µl plasma, respectively, after 30 min (Figure 5(A)). Similarly, assay of the total content of each of these following reduction of the plasma showed significant increases in all of them over the collection period, resulting in levels of 17·8 ($SO_3^{2-}$), 35·0 (cys) and 4·4 (GSH) nmol/100 µl plasma.

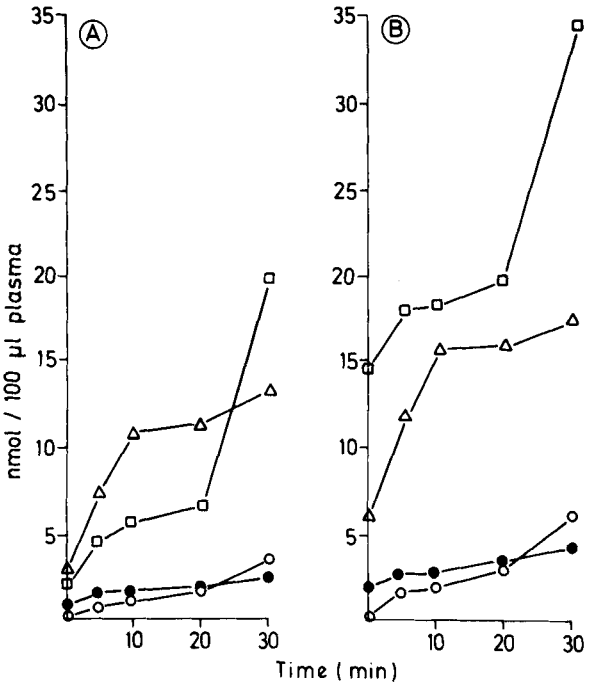


Figure 5. Hepatic portal vein free (A) and total (B) thiol and sulphite levels following intraintestinal administrtaion of ($^{14}$C)-NAC to rats: ○——○ NAC; ●——● GSH; □——□ Cysteine; △——△ $SO_3^{2-}$. Data typical of four individual experiments

Radiometric analysis of fractions obtained from the thiol analysis of plasma obtained 30 min after a dose of [$^{14}$C]-NAC demonstrated the presence of radioactivity in both the NAC–mBBr adduct peak and that of cys–mBBr, with low levels of $^{14}$C also associated with the corresponding GSH adduct peak (Figure 6).

## DISCUSSION

Incubations of isolated intestinal epithelial cells were shown to rapidly metabolize [$^{14}$C]-NAC to [$^{14}$C]-cysteine. Both endogenous and metabolically derived cysteine were evenly distributed among the aqueous phase of the incubation, indicating that this amino acid is freely permeable to the epithelial cell plasma membrane. Additionally, neither free NAC nor disulfide-bound NAC could be detected intracellularly, indicating that either NAC is metabolized at the plasma membrane surface, or NAC is metabolized extremely rapidly when entering the intracellular space.

Both NAC and cysteine were shown to undergo oxidation to disulfide

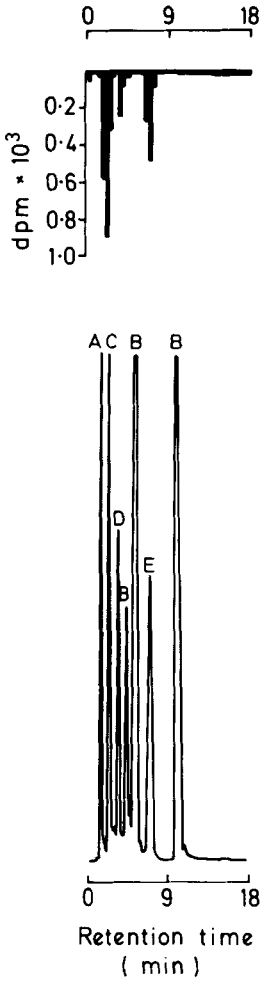


Figure 6. Radiochromatographic h.p.l.c. traces obtained from the assay of the thiol and sulfite content of hepatic portal vein plasma 30 min after an intraintestinal dose of ($^{14}$C)-NAC (10 mg; 25 μCi). Peak assignment and integrator attenuation as Figure 1

species under the conditions of incubation, but it is probable that this arose chemically.

It is well established that cysteine plays a pivotal role in the biochemical functioning of the cell, serving, among other things, as a source of inorganic sulfur through the β-mercaptopyruvate pathway and as an obligate precursor to GSH biosynthesis. The inclusion of NAC in cellular incubations, with subsequent metabolism to cysteine, failed to produce inorganic sulfite in the incubations, and did not stimulate novel intracellular GSH biosynthesis. It may be that in the former case the cells lack one or more of the enzymes

responsible for the multistep conversion of cysteine to $SO_3^{2-}$ or that the cells do indeed produce $SO_3^{2-}$ but continue to oxidize it to inorganic sulfate, which is not detected by our assays. In the case of GSH, it may be that glycine and/or glutamate, cosubstrates for GSH biosynthesis, are washed out of the cells during their preparation.

Extending these studies, a measure of the rate and extent of intestinal metabolism of NAC *in vivo* comes from the demonstration that despite a relatively high dose of NAC (10 mg) administered intraintestinally, the levels of NAC (free and oxidized) in the hepatic portal vein plasma are low in comparison to those of its primary metabolites.

The deacetylation of NAC by the small intestine was confirmed *in vivo* by the incorporation of $^{14}C$ from [$^{14}C$]-NAC into increased levels of hepatic portal vein plasma free cysteine. Unlike the isolated cell incubations, further metabolites of NAC-derived cysteine were detected in the plasma. The free sulfite levels were shown to dramatically increase in the plasma following NAC dosing. It is tempting to speculate that this sulfite resulted from metabolism of cysteine derived within the lumenal epithelium, through the β-mercaptopyruvate pathway in sub-epithelial areas of the gut.

In addition to this, and most importantly, hepatic portal vein plasma levels of free GSH were shown to increase significantly following NAC dosing. Experiments with [$^{14}C$]-NAC also demonstrated incorporation of $^{14}C$ into this GSH. The site of synthesis of this 'extra' GSH is uncertain. It is unlikely to be synthesized in the intestine itself, but is more likely to result from stimulated hepatic biosynthesis and release of GSH as a consequence of the supply of NAC-derived cysteine in the hepatic portal circulation. This interesting aspect of the metabolism of NAC is being investigated further, particularly with respect to possible therapeutic implications.

Thus, in summary, the apparent extensive first-pass effects and resultant low bioavailability of orally administered NAC in humans[1] may, at least in

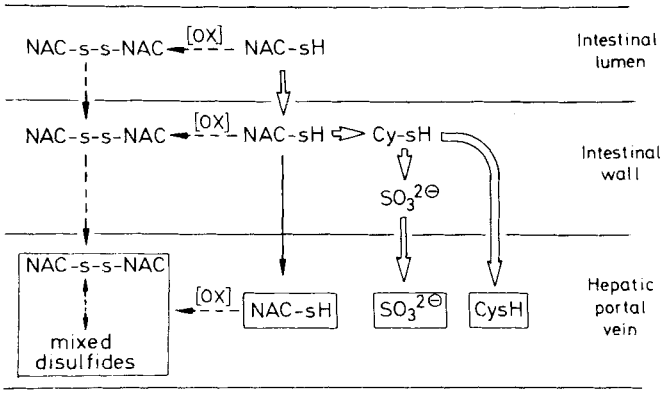


Figure 7. A schematic representation of the intestinal metabolism of NAC in the rat

part, be explained by extensive metabolism of NAC in the small intestine to both cysteine and sulfite. These observations may also provide the basis for clarification of the mechanism(s) of hepatoprotection of NAC in cases of paracetamol overdosage both *in vitro*[4] and *in vivo*[5]. N-acetylcysteine is known to be a precursor of hepatocellular GSH and sulfate *in vivo*[5] during depletion of these components by reactive paracetamol metabolites, and is generally thought to do so by its direct metabolism to cysteine in the liver itself. In view of the poor systemic bioavailability of NAC when given orally and the results obtained here, it may be that the origin of this protective effect of NAC lies in extensive first-pass metabolism of NAC to cysteine and sulfite in the intestines, with immediate supply of these metabolites to the liver via the hepatic portal circulation.

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