incubation. In neither case was enhancement of toxicity observed. This inability to detect enhancement of cytotoxicity by caffeine is contrary to the previous report using neocarzinostatin in L1210 cells [13]. We therefore applied neocarzinostatin to our L1210 cells for 1 hr followed by post-incubation in caffeine-containing medium. Again, no enhancement of toxicity was observed. The only reason offered for this discrepancy is that significant differences exist between our cell lines and that previously reported. We must conclude that resistance is not related to a caffeine-sensitive pathway in our cells.

The present study characterizes a system in which an L1210 subline is specifically resistant to certain *cis*-platinum coordination complexes. Several *cis*-platinum complexes have been shown previously to be equitoxic to these two cell lines [2, 14]. These equitoxic compounds have a bulky group attached to the amines. One such example, 1,2-diaminocyclohexanedichloroplatinum(II), is now being considered for clinical trials in the hope that it will overcome resistance. This communication shows, however, that various mechanisms of resistance may exist and raises the possibility that a patient who had been treated previously with *cis*-DDP may have developed or may develop tumor cells that are resistant to the diaminocyclohexane derivative.

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Sulfate depletion after acetaminophen administration and replenishment by infusion of sodium sulfate or *N*-acetylcysteine in rats

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Humans and animals have a limited capacity to convert phenolic drugs to their sulfate conjugates [1–6]. The formation of sulfate conjugates of such drugs can be increased by administration of inorganic sulfate or of a sulfate donor substance, suggesting that the capacity-limiting factor is the availability of free sulfate in the body rather than the activation of sulfate or the transfer of the activated sulfate to the drug [1, 3, 6, 7]. This interpretation was questioned recently because the serum concentration of inorganic ("free") sulfate in rats decreased only slightly (from 0.92 to 0.71 mM) after intravenous injection of phenol (266 μmoles/kg) [8]. A study was initiated therefore to determine the effect of various doses of acetaminophen on the concentration of free sulfate in the serum of rats.

Acetaminophen is eliminated from the body primarily by conversion of the drug to glucuronide and sulfate conjugates [4]. In parallel with these processes, there also occurs formation of a quantitatively minor but highly reactive metabolite of acetaminophen that can cause serious and sometimes fatal hepatotoxicity following overdoses of the drug [9]. Because the processes are parallel and therefore competing, impaired formation of acetaminophen con-

jugates can be expected to be associated with increased formation of reactive metabolite and therefore with increased toxicity. This has been demonstrated experimentally [10]. Conversely, enhanced conjugation of acetaminophen should result in decreased formation of the reactive metabolite and therefore in decreased toxicity. This, too, has been confirmed experimentally in that administration of sodium sulfate was found to decrease the lethality of acetaminophen in mice [11]. *N*-Acetylcysteine, a clinically accepted antidote for acetaminophen [12], also increases the conversion of the drug to its sulfate conjugate [13]. It is of interest therefore to compare the efficacy of sodium sulfate and *N*-acetylcysteine for replenishing free sulfate in the body.

Male Sprague–Dawley rats weighing 320–370 g received a single i.v. injection af acetaminophen, either 15, 30, 150 or 300 mg/kg (0.1 to 2 mmoles/kg). Food, but not water, was withheld during the study. Venous blood samples (0.4 ml) were collected immediately before, and periodically after, the injection as described previously [6, 13]. Serum was separated and assayed for free sulfate by a turbidimetric method [14] that was scaled down to a sample volume of 0.15 ml. The pre-acetaminophen serum free-sulfate concentration in thirty-two animals was 0.89 ± 0.08 mM (mean ± S.D.). This concentration decreased substantially after acetaminophen administration, the magnitude and duration of the effect being a function of the acetaminophen dose (Fig. 1). Similar results were obtained upon intravenous administration of salicylamide in single doses of 30–300 mg/kg (unpublished data). That drug is also subject to capacity-limited conjugation with sulfate [1, 15].

In another experiment, acetaminophen was administered to the rats as a single i.v. dose of 30 mg/kg, followed 2 hr later by an infusion (in saline solution) of 16 $mg \cdot rat^{-1} \cdot hr^{-1}$, or about 0.3 $mmole \cdot kg^{-1} \cdot hr^{-1}$, for 8 hr. Some of the animals received infusions that also contained either sodium sulfate or *N*-acetylcysteine at concentrations such that the animals received these compounds at a rate of about 0.5 $mmole \cdot kg^{-1} \cdot hr^{-1}$ and 1.8 $mmoles \cdot kg^{-1} \cdot hr^{-1}$, respectively. Administration of acetaminophen alone resulted in a pronounced decline of serum free-sulfate concentrations (Fig. 2). Concomitant administration of sodium sulfate or *N*-acetylcysteine prevented this decline and actually resulted in a gradual increase of serum free sulfate to superphysiologic concentrations (Fig. 2).

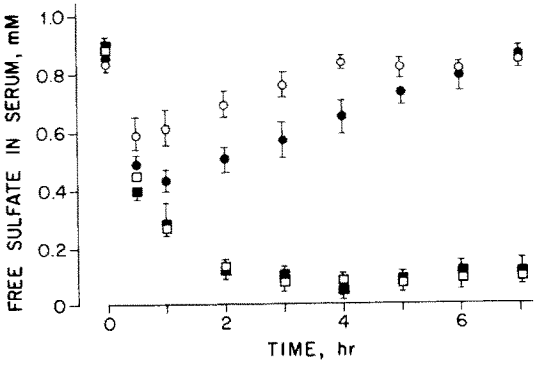

Fig. 1. Effect of acetaminophen administration on concentrations of free sulfate in serum of rats. The drug was injected i.v. at zero time as a single dose of either 15 (○), 30 (●), 150 (□) or 300 (■) mg/kg body weight. Data are mean values; N = 5–7. Vertical bars represent one S.E.M.

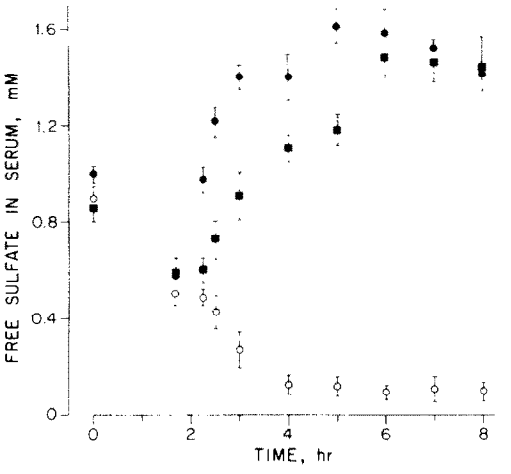

Fig. 2, Effect of i.v. infusion (started at 2 hr) of sodium sulfate or *N*-acetylcysteine on serum free-sulfate concentrations in rats after sulfate depletion by injection (at zero time) and infusion (started at 2 hr) of acetaminophen. Key: (○) control animals (acetaminophen only); (●) sodium sulfate; (■) *N*-acetylcysteine. Results are shown as means ± S.E.M.: N = 3.

Incubation of either citrated rat blood or serum with a high concentration (24 mM) of *N*-acetylcysteine for 1 hr at 37° had no significant effect (<10 per cent) on the concentrations of free sulfate. A 24 mM solution of *N*-acetylcysteine in water contained 0.04 mM apparent free sulfate and this concentration did not increase during incubation.

It is known that hepatic biotransformation of cysteine leads to formation of inorganic sulfate [16]. Consistent with this bioconversion is the delay, relative to the results during sodium sulfate administration, of serum free-sulfate accumulation during *N*-acetylcysteine infusion (Fig. 2). The latter agent also appears to be a poorer sulfate donor than sodium sulfate itself under the experimental conditions (considering that the amino acid infusion rate was 3.6 times that of sodium sulfate).The antidotal efficacy of *N*-acetylcysteine in acetaminophen intoxication may not be due only to its sulfate donor properties; its ability to prevent glutathione depletion and thereby to enhance inactivation of the reactive metabolite of acetaminophen [17] may be equally or more important.

The results of this investigation demonstrate directly that (a) acetaminophen administration can cause depletion of free sulfate in the body, (b) *N*-acetylcysteine, an effective antidote for acetaminophen poisoning, can prevent such depletion by acting as a sulfate donor, and (c) replenishment of serum free sulfate is effected more rapidly by i.v. infusion of sodium sulfate than of *N*-acetylcysteine

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The effect of organophosphate poisoning on plasma cyclic AMP in rats

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Cyclic adenosine monophosphate which is known as molecule confined to regulation of protein kinase activity, is present in all types of mammalian cells. It is generated via activation of intracellular adenylate cyclase and released to the extracellular fluid [1–7]. Cyclic nucleotide change in the plasma and urine has been reported to occur in response to various hormonal deficiences and treatments [8–11]. Thus, the increase in plasma cAMP was found to follow stimulation of β-adrenergic receptors by endogenous catecholamines [11] as well as administration of glucagon and isoprenaline [6]. Dramatic increase in plasma cAMP following poisoning with soman, has been revealed by Stitcher *et al.* [12] and interpreted as to be caused by the ACh-induced release of humoral and pharmacologically active substances which induce cAMP-generation; the cAMP participates in anti-ChE poisoning by enhancing the concentration of ACh in the brain or by enhancing the release of ACh at the neuromuscular junctions [13].

While the role played by cAMP in anti-ChE poisoning is within the scope of speculation, the plasma cAMP, being in a dynamic steady-state relation with its intracellular pools [7], could be used for assessment of the effect of poisoning on the cAMP-mediated cellular processes. In this context we studied the dependence of plasma cAMP level on the type and dose of organophosphate along with the effect of antidote on the restoration of the poison-induced rise in plasma cAMP and inhibition of RBC AChE activity.

Male albino rats, weighing 190–220 g were used after a 18–20 hr fast. Animals were poisoned by subcutaneous administration of 0.9–1.2 LD_{50} soman or 0.3–1.2 LD_{50} VX. At the onset of convulsions, 10–15 min after poisoning, one out of four animal subgroups received saline, whereas the others received intramuscular injections of (1) HI-6, 50 mg/kg, (2) atropin, 10 mg/kg, (3) the mixture of atropin 10 mg/kg and HI-6,5/mg/kg. The same number of non-poisoned, control animals were divided into four subgroups and treated according to the same protocol as the poisoned ones.

Untreated or saline treated rats were decapitated at the onset of convulsion phase, 10–15 min following poisoning, the others, 1 hr after HI-6 or atropin administration. Blood aliquots (2 ml) were collected in centrifuge tubes containing 20 μl of 0.5 M EDTA, pH 7.5 and centrifuged. The cAMP was determined in blood plasma by radioimmuno assay method. The cAMP assay kit was Radiochemical Centre (Amersham, U.K.) product. The AChE activity in erythrocytes was estimated by Michel method [23] using acetyl-β methylcholine chloride as substrate.

In plasma of male rats the cAMP level was approx 20 pmol/ml (Table 1), whereas in females the concentrations were two-fold higher (data not shown). Depending on sex of animals, the reported values ranged from 14 to 37 pmol/ml [11, 14, 18]. Thus, in plasma of female rats Patterson *et al.* [18] found 37 pmol/ml, while for the male rats Sarkar *et al.* [15] and Turinski [14] reported 14 and 20 pmol/ml, respectively. Circadian variations of plasma cAMP, which has been demonstrated to exist [19] may also contribute to a large variations of normal values.

Abbreviations used: cAMP, cyclic adenosine monophosphate; ACh, acetylcholine; AChE, acetylcholinesterase (E.C.3.1.1.7); RBC, red blood cells; VX, O-ethyl-S-(2-diisopropylaminoethyl)methylphosphotioate; soman, pinacolyl methylphosphonofluoridate; HI-6,1-(2-hydroxyiminomethyl-1-pyridinio-3-(4-carbamoyl-1-pyridinio)-2oxapropane dichloride.