and that [^{3}H]PCP may be binding to this channel. It is noteworthy that these calcium antagonists also block α_1-adrenergic and muscarinic acetylcholine receptors in the same concentration range in which they inhibit [^{3}H]PCP binding [14, 15], particularly since occupation of α-adrenergic and muscarinic receptors is frequently coupled to changes in calcium permeability [16, 17]. Although little is known structurally about other channels permeable to calcium, the acetylcholine receptor-ionic channel complex from *Torpedo* membrane has been purified to homogeneity and has been well characterized [18]. Hence, the ionic channel associated with the acetylcholine receptor may provide a useful system for analysis of the precise mechanism by which calcium channel antagonists interact with a receptor–channel complex.

In sum, we have shown that the specific binding of [^{3}H]PCP to electric organ membrane isolated from *T. californica* was displaced by $CaCl_2$ and several structurally different classes of calcium channel antagonists, including verapamil, the diphyenlmethylalkylamines, and the 1,4-dihydropyridines. The calcium antagonists appeared to inhibit [^{3}H]PCP binding competitively. The IC_{50} values for displacement of [^{3}H]PCP binding by these compounds were: $CaCl_2$, 2.3 mM; verapamil, 4.8 μM; flunarizine, 2.7 μM; cinnarizine, 1.5 μM; nicardipine, 1.4 μM; nimodipine, 2.5 μM; and nifedipine, 9.8 μM. These studies suggest that calcium channel antagonists may interact with the ion channel associated with the nicotinic acetylcholine receptor.

Addendum—Subsequent to the submission of this manuscript, a detailed analysis of the effects of calcium on [^{3}H]PCP binding to acetylcholine receptor enriched membrane from *Torpedo* electric organ appeared [19]. It was found that, in the presence of 0.2 mM carbachol, calcium decreases the binding of [^{3}H]PCP with an $IC_{50} \simeq 1$ mM. The inhibition of [^{3}H]PCP binding by calcium results from a decrease in the equilibrium affinity for PCP in the presence of carbachol.

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Identification of 6-mercaptopurine riboside in patients receiving 6-mercaptopurine as a prolonged intravenous infusion

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6-Mercaptopurine (6-MP) is an analog of hypoxanthine that has been in clinical use for over 30 years [1]. Its value as a remission maintenance agent for the treatment of acute lymphoblastic leukemia has been well established [2]. To be biologically active, 6-MP must first undergo intracellular conversion to a nucleotide, thioinosinic acid (TIMP) [3]. This compound is then converted to thiguanine ribonucleotide and deoxyribonucleotide, and these compounds have been shown to subsequently be incorporated into RNA and DNA [4]. Following administration of 6-MP in man, the drug undergoes extensive metabolism initiated by the enzyme xanthine oxidase. The major product of this pathway is 6-thiouric acid (TU) with 6-thioxanthine (TX) being an intermediary metabolite [3, 5]. Methylation of 6-MP has also been shown to occur in man [5]. Following metabolism, 6-MP and its catabolites are eliminated in the

urine. Previous studies of the urinary metabolite pattern of 6-MP in man have demonstrated that TU is the major compound in urine following oral administration, while after intravenous dosing unchanged 6-MP and TU are the major metabolites [5–7].

We are administering 6-MP as a prolonged intravenous infusion in an ongoing clinical trial and have isolated and identified a previously unreported metabolite, 6-mercaptopurine riboside (MPR), in the urine of eight patients. The methods used to confirm the identify of this new metabolite and its possible significance are described below.

Materials and methods

Patients. The patients studied were receiving 6-MP as an intravenous infusion at a dose rate of 50 mg/m²/hr for 12–48 hr on an approved Phase I trial. The median age of the patients studied was 10 years (range 3–17), and all had refractory acute lymphoblastic leukemia. Informed consent was obtained from all patients or their parents. All urine was collected during the infusion and for 24 hr post infusion.

Materials. Reference standards for MP, TX, and MPR were obtained from the Sigma Chemical Co., St. Louis, MO. Thiouric acid and 8-OH-6-MP standards were provided by Dr. Gertrude Elion, Wellcome Research Laboratories, Research Triangle Park, NC 27709.

High performance ligand chromatography (HPLC) assay for 6-MP and metabolites. Twenty microliter urine samples were injected directly onto the HPLC system following filtration through 0.22 μm filters (Millipore). A Beckman C-18 column (4.6 mm × 25 cm) with 5 micron size particles was utilized to separate the various compounds of interest. The mobile phase consisted of 1% acetonitrile, 0.2% acetic acid, and 98.8% water and was run isocratically at a flow rate of 1 ml/min. The effluent was monitored with a Waters dual channel u.v. detector at 313 nm and 340 nm, 0.01 AUFS. Under these conditions, there was complete separation between the four compounds of interest. The authentic standards for TU, 6-MP, TX, and MPR had retention times of 9.9, 12.5, 16.8, and 24.5 min, respectively, with 8-OH-6-MP essentially co-eluting with TU in urine samples.

Isolation of MPR from human samples. MPR was isolated and collected from human urine by HPLC using an ODS column with repetitive collection of the effluent under the MPR peak as it eluted from the HPLC. The MPR-containing effluent was then concentrated by lyophilization for subsequent studies.

Enzymatic identification of metabolite. Separate experiments were conducted with the authentic standard of MPR and with the metabolite isolated from the urine of patients treated with 6-MP. Either MPR or the metabolite was incubated with purine nucleoside phosphorylase (orthophosphate ribosyltransferase; EC 2.4.2.1) (PNP) and 0.05 M phosphate buffer, pH 7.4, for 5 min at 37°. Reactions were stopped by placing the samples on ice. Aliquots of the samples were injected onto the HPLC prior to and following incubation with PNP to demonstrate conversion of both compounds to 6-MP.

Gas chromatography/mass spectrometry (GC/MS). MPR which was isolated from human urine by HPLC was identified by GC/MS after forming its *S*-methyl trisilyl derivative. HPLC collections of the metabolite were reacted with diazomethane in ether to form the S-CH_3 derivative. After removing the solvent under a stream of dry nitrogen, the residue was reacted with *N,O*-bis (trimethylsilyl) acetamide to form the *S*-methyl trisilyl derivative. Identification of the metabolite was accomplished using a Ribermag R-10-10-C mass spectrometer equipped with a Girdel series 32 gas chromatograph (Delsi Nermag, Houston, TX. The mass spectrometer was operated in the chemical ionization mode using methane at a source pressure of 0.5 torr as the reactant gas. Separation of compounds was achieved with a 30 m × 0.75 mm i.d. glass capillary column coated with a 1.0 μm film of SE-54 (Supelco, Inc., Bellefonte, PA). The column temperature was 240°, and methane at 10 cc/min was used as both the carrier and reagent gas for chemical ionization. Under these conditions, the metabolite had a retention time of 8 min.

Results and discussion

Complete urine collections were obtained from eight patients who received 6-MP intravenous infusions ranging from 12 to 48 hr. in duration. A typical high performance liquid chromatogram of a urine sample obtained during a 6-MP infusion is shown in Fig. 1. In addition to 6-MP, TU, and TX, a peak was observed which was absent from patient urine samples obtained prior to 6-MP administration and was found to co-elute with authentic MPR. In addition, the relative u.v. absorbance measured at 313 nm and 280 nm was similar for reference MPR and metabolite (data not shown).

PNP is an enzyme known to convert purine nucleosides to their respective bases [8, 9]. Following incubation with PNP, MPR is converted to 6-MP. When, in separate experiments, authentic MPR and the isolated urinary metabolite were incubated with PNP, the MPR and metabolite peaks disappeared and a new peak that co-eluted with and had an identical u.v. absorption spectrum with 6-MP appeared (Fig. 2). These enzymatic peak-shifting experiments suggest that the metabolite isolated from the urine of the patients was MPR.

Chemical ionization mass spectra of the *S*-methyl trimethylsilyl derivatives of reference MPR (Fig. 3A) and the 6-MP metabolite isolated from human urine by HPLC (Fig. 3B) were obtained to confirm the identify of the metabolite. Both compounds exhibited an intense protonated molecular ion at *m/z* 515 and the characteristic adduct ion ($M + C_2H_5^+$) was observed at *m/z* 543. These results are similar to that for the chemical ionization spectrum of permethylated MPR [10]. In addition, the characteristic

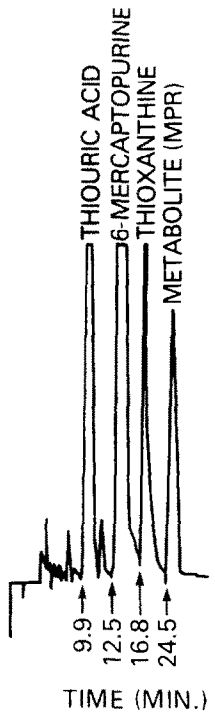

Fig. 1. HPLC elution profile of a 20-μl urine sample obtained from a patient during an intravenous infusion of 6-MP. Effluent u.v. absorbance was monitored at 313 nm. The various urinary metabolites with their respective retention times are noted.

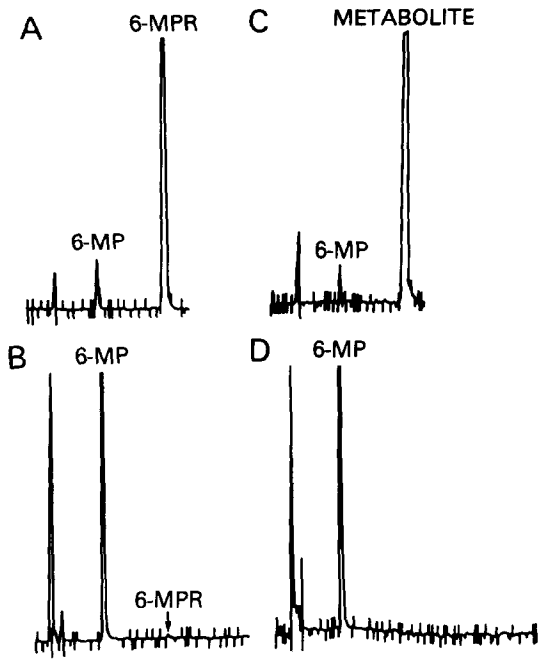

Fig. 2. Conversion of authentic MPR and urinary metabolite to 6-MP by PNP. Authentic MPR (A and B) and the metabolite (C and D) isolated from human urine were incubated with 78 mU of PNP for 5 min at 37° in separate simultaneous experiments. A and C are the pre-incubation and B and D are the post-incubation HPLC elution profiles.

ion ($MH^+ - 15$) for silylated derivatives was observed at m/z 499. Loss of the sugar moiety from MPR resulted in the ion observed at m/z 167 ($B^+ + 15$) and is the protonated S-CH_3 derivative of 6-MP. The prominent ion fragment observed at m/z 259 ($M^+ - 255$) probably represents the sugar minus CH_3SiOH. For a detailed analysis of the fragmentation patterns of trimethylsilyl derivatives of nucleosides, see the discussion by Pang *et al.* [11].

The above results provide strong evidence that the metabolite we have identified in the urine of patients receiving 6-MP is MPR. Although 59% of the administered dose of 6-MP was eliminated in the urine as parent compound or metabolites within 24 hr of completion of the intravenous infusion, MPR accounted for only 1.5 ± 0.4% (N = 8) of the administered dose. This indicates that MPR was a quantitatively minor metabolite of 6-MP. MPR has not been detected previously in patients receiving 6-MP on conventional low dose oral administration schedules, probably since these patients achieve much lower plasma 6-MP levels [12]. The patients in this study received relatively high doses of 6-MP for prolonged infusion periods. It is possible that only patients receiving high dose, parenteral 6-MP therapy may produce enough MPR to be detected by HPLC techniques, and, therefore, this difference in dose may explain why this compound has not been reported as a metabolite of 6-MP in previous studies.

Although a minor metabolite of 6-MP, MPR may be of clinical importance. The known metabolites of 6-MP, such as TU and TX, have no anti-neoplastic activity. In contrast, MPR, the nucleoside anabolite of 6-MP, has known anti-neoplastic activity [13, 14]. There are two possible pathways for its *in vivo* formation. One is by direct addition of a ribose onto the 6-MP molecule, a reaction mediated by PNP and requiring the presence of ribose-1-phosphate. The other pathway is by conversion of 6-MP to 6-thioinosinate (TIMP), a reaction mediated by the enzyme hypoxanthine-

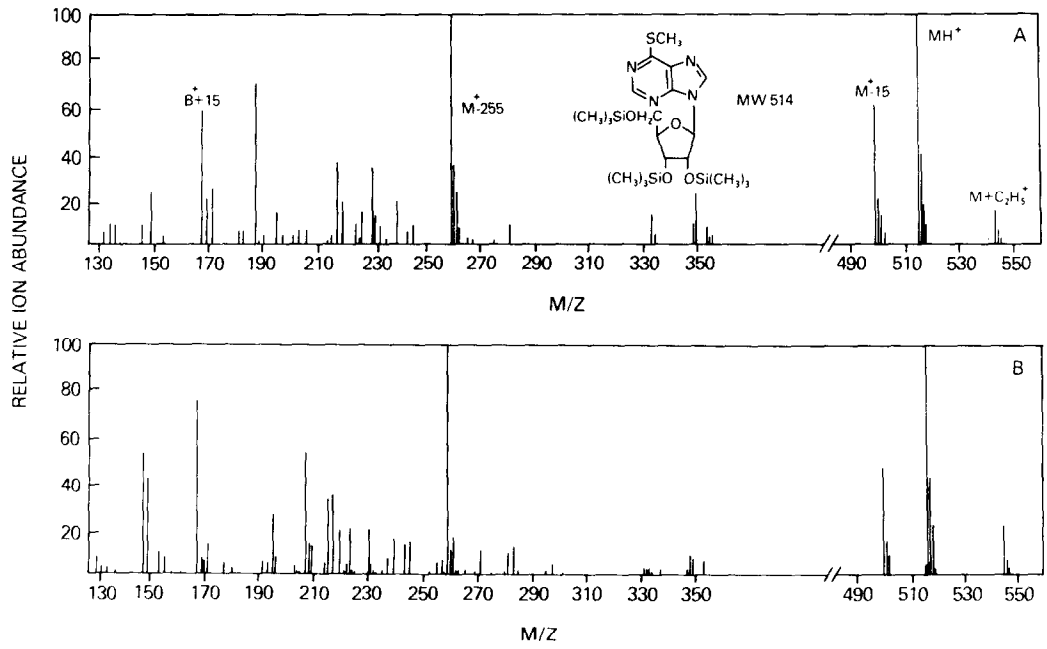

Fig. 3. Chemical ionization mass spectra of the authentic *S*-methylated, trisilyl derivative of MPR (A) and the *S*-methylated, trisilyl derivative of putative MPR isolated from human urine (B).

guanine phosphoribosyltransferase, and requiring the presence of phosphoribosyl-1-pyrophosphate. The intracellular 6-MP nucleotide, TIMP, may then subsequently, be broken down to the nucleoside, MPR, by a phosphatase. The MPR which then appears in the urine may be an indirect marker for intracellular conversion of 6-MP to TIMP. The latter pathway has been proposed as the mechanism by which allopurinol riboside has been found in the urine of a PNP-deficient patient receiving allopurinol therapy [15].

In summary, we have identified a previously unreported metabolite of 6-MP, 6-mercaptopurine riboside, in the urine of patients receiving 6-MP as a prolonged intravenous infusion. Although MPR is a minor metabolite of 6-MP, accounting for less than 2% of the administered dose of 6-MP, it may be of clinical significance. Unlike the previously reported urinary metabolites of 6-MP, MPR has known anti-neoplastic activity. In addition, if its formation results from phosphatase-mediated breakdown of TIMP, then MPR may be a marker of intracellular activation of 6-MP.

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Neonatal chlordecone alteration of the ontogeny of sex-differentiated hepatic drug and xenobiotic metabolizing enzymes*

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The perinatal period of development in humans and rodents is recognized as a time during which critical organizational events are still taking place in the central nervous system. Organizational effects are permanent and the expression of this type of hormone regulation does occur until after the onset of sexual maturation, long after the effector has been metabolized and excreted. The fetus and newborn are, therefore, particularly susceptible to hormone imbalance that may be brought about by genetic endocrine disorders, abnormal pregnancies, drug treatment or exposure to environmental chemicals that can change the hormonal milieu during this critical period of development.

Previous work by us and others has demonstrated the role of neonatal androgen and estrogen for the determination of adult sex-differentiated hepatic metabolism [1–5]. We have shown that perinatal exposure to hormones and estrogenically-active xenobiotics can alter the ontogeny of hepatic metabolism [5–7]. Chlordecone is an estrogenically-active and toxic chemical [8–11] that selectively alters brain and pituitary endorphin levels in prepubertal and adult rats following neonatal exposure [12]. In this study we have investigated the potential of chlordecone when administered during the neonatal period to alter the ontogeny of the following drug and xenobiotic metabolizing enzyme systems: benzo[*a*]pyrene hydroxylase, glutathione *S*-transferase, UDP-glucuronyltransferase and cytochrome P-450 content.

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Materials and methods

Experiments were carried out using Sprague–Dawley CD rats (Charles River Breeding Laboratories Inc., Wilmington, MA). Animals had free access to food (Purina Lab Chow 5001) and water. The animals were housed in a controlled environment (21°; 12 hrs light–dark cycle), were weaned at 21–23 days of age, and were housed four animals per cage after weaning. Chlordecone (Kepone; 99% pure)