Molecular Cloning of the Alcohol/Hydroxysteroid Form (mST_{al}) of Sulfotransferase from Mouse Liver[1,2]

Ah-Ng Tony Kong,[3,5] Deling Tao,[3,4] Meihui Ma,[3] and Linding Yang[3]

Received October 13, 1992; accepted November 12, 1992
KEY WORDS: sulfotransferases; sulfation; drug metabolism; alcohol sulfotransferase; Phase II metabolism.

INTRODUCTION

Sulfotransferases (STs)[6] are Phase II drug metabolizing enzymes that sulfoconjugate a variety of endogenous and exogenous compounds such as biogenic amines (e.g., catecholamines), steroid hormones (i.e., androgens), bile acids (e.g., hyodeoxygholic acid), drugs (e.g., propranol and acetaminophen), and carcinogens [e.g., *N*-hydroxy-2-acetylaminofluorene and hydroxylated 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD)] (1,2). STs also play an important role in the biosynthesis of proteoglycans, which confer important recognition properties on cell surfaces that influence cell adhesion and migration as well as the binding of growth factors and enzyme inhibitors (3).

STs belong to a multigene family which comprises at least six genes in the rat (4–9), one in bovine (10), two in the mouse (11), and one of human origin (12,13). These conjugating enzymes catalyze the coupling of sulfate ($SO_3^{=}$) from 3′-phosphoadenosine-5′-phosphosulfate (PAPS) to molecules possessing phenols, enols, alcohols, or amines to form mono esters of sulfuric acid or sulfate esters (14). Conjugation with sulfate confers greater polarity and water solubility on the parent agents, thereby facilitating biliary and/or urinary excretion and detoxification.

ST-a catalyzes O-sulfation of carcinogenic and noncarcinogenic exogenous alcohols, as well as endogenous steroidal alcohols, such as dehydroepiandrosterone (DHEA) (1). Regulation of the metabolism of the naturally occurring steroid DHEA has been implicated in a variety of disease processes such as aging, diabetes, autoimmune diseases, cancer, obesity, and atherosclerosis (15). Unlike primates, rodents do not synthesize DHEA from cholesterol, and thus its effects can be investigated by administering as a supplement to the diet. Such studies have shown DHEA to have remarkable chemopreventive effects on the above disorders. DHEA has also been found to affect the expression of cellular cytochrome P450s, thereby influencing the balance of metabolic activities associated with the initiation phase of chemical carcinogenesis and/or toxicity (15). Hence, examining the regulation of ST-a, one of the major enzymes that metabolize DHEA, should provide important insights toward our understanding of the molecular basis for the regulation of sulfate conjugation of steroid hormones in humans and in rodents. Here we report the molecular cloning of mST_{al} cDNA from mouse liver, which provides an important first step toward unraveling its physiological function and regulation of drug and carcinogen metabolism at the molecular level.

MATERIALS AND METHODS

Screening of a λZAP cDNA Library

A B6CBA female mouse liver cDNA library in phage LAMBDA-ZAP Vector and the host cells (*E. coli* strains, BB4 and XL-1 Blue) were purchased from Stratagene (La Jolla, CA). A total of 600,000 plaques at a density of ~30,000 per 150-mm plate was screened using a 655-bp *Bam*HI/*Nco*I fragment of a rat ST_a cDNA according to methods established in our laboratory (13) and by Ausubel *et al.* (16). The rat ST_a cDNA was obtained via polymerase chain reaction (PCR) using specific oligonucleotides designed from published rat cDNA sequences (5) as described previously (13). The probes were prepared by random hexamer-primed synthesis (Prime-a-Gene Labeling System; Promega, Madison, WI) using [α-^{32}P]dCTP (3000 Ci/mmol; NEN Dupont; 1 Ci = 37 GBq) to generate a specific activity of 1–3 $\times$ 10^9 cpm/μg DNA (17). Positive plaques were replated and rescreened twice until five isolated positive plaques were selected for *in vivo* excision of pBluescript SK vector with the cDNA inserts using R408 helper phage, according to protocols provided by the manufacturer (Stratagene). The clones were purified and four of the clones showed positive hybridization on Southern blotting. One of the clones, 1-1, was further characterized by sequencing its entire cDNA in both directions by the dideoxynucleotide chain termination method of Sanger (18), modified as in Sequenase Version 2.0 protocols (USB).

RESULTS AND DISCUSSION

Plaque screening of the mouse liver LAMBDA-ZAP cDNA library (6 $\times$ 10^5 independent clones) with the radiolabeled rat ST_a cDNA probes identified five ST_a cDNA clones. One of the clones, 1-1, has 1004 bp and is designated mST_{al}. Figure 1 shows the nucleotide sequence together with

---

[1] The sequence data in this paper have been submitted to the EMBL/Genbank Data Library under the accession number L02335.

[2] Presented in part at the 7th Annual Meeting of the American Association of Pharmaceutical Scientists (AAPS), San Antonio, Texas, November 15–19, 1992.

[3] Division of Clinical Pharmacology, Department of Medicine, Thomas Jefferson University, 1100 Walnut Street—Room 601, Philadelphia, Pennsylvania 19107-5563.

[4] Department of Biology, Eastern Michigan University, Ypsilanti, Michigan 48197.

[5] To whom correspondence should be addressed.

[6] ST(s) is used to denote sulfotransferase protein(s) and italic *ST* with subscript, e.g., ST_a, indicates the gene that encodes the protein.

0724-8741/93/0400-0627$07.00/0 © 1993 Plenum Publishing Corporation

```
  1                                                                  GAATTCCCTTACAAGGAGTTATTCATG
 25   ATG ATG TCA GAC TAT AAT TGG TTT GAA GGC ATA CCT TTT CCT GCC ATA TCA TAT CAA AGA
  1   Met Met Ser Asp Tyr Asn Trp Phe Glu Gly Ile Pro Phe Pro Ala Ile Ser Tyr Gln Arg

 85   GAA ATT TTG GAA GAT ATT CGT AAT AAG TTT GTG GTG AAA GAA GAA GAC TTG TTG ATA TTA
 21   Glu Ile Leu Glu Asp Ile Arg Asn Lys Phe Val Val Lys Glu Glu Asp Leu Leu Ile Leu

145   ACT TAC CCC AAG TCA GGA ACG AAC TGG CTG ATT GAG ATT GTA TGC TTG ATT CAG ACC AAG
 41   Thr Tyr Pro Lys Ser Gly Thr Asn Trp Leu Ile Glu Ile Val Cys Leu Ile Gln Thr Lys

205   GGA GAT CCG AAG TGG ATC CAA ACT GTG CCC ATT TGG AAC CGC TCA CCC TGG ATA GAG ACT
 61   Gly Asp Pro Lys Trp Ile Gln Thr Val Pro Ile Trp Asn Arg Ser Pro Trp Ile Glu Thr

265   GAT ATA GGA TAT TCT GCA TTA ATC AAT AAG GAA GGA CCA CGA CTC ATA ACC TCC CAT CTT
 81   Asp Ile Gly Tyr Ser Ala Leu Ile Asn Lys Glu Gly Pro Arg Leu Ile Thr Ser His Leu

325   CCC ATC CAT CTC TTC TCC AAG TCT TTC TTC AGT TCC AAG GCC AAG GCG ATC TAT CTC GTG
101   Pro Ile His Leu Phe Ser Lys Ser Phe Phe Ser Ser Lys Ala Lys Ala Ile Tyr Leu Val

385   AGA AAT CCC AGA GAT ATT CTT GTG TCT GGT TAC TTT TTC TGG GGT AAC ACA AAC CTT GTG
121   Arg Asn Pro Arg Asp Ile Leu Val Ser Gly Tyr Phe Phe Trp Gly Asn Thr Asn Leu Val

445   AAG AAT CCA GGG TCA CTC GGA ACT TAT TTT GAA TGG TTC CTC AAA GGA AAT GTT CTA TTC
141   Lys Asn Pro Gly Ser Leu Gly Thr Tyr Phe Glu Trp Phe Leu Lys Gly Asn Val Leu Phe

505   GGA TCA TGG TTT GAG CAT GTT CGT GGC TGG CTG TCC ATG AGA GAA TGG GAC AAC TTT TTG
161   Gly Ser Trp Phe Glu His Val Arg Gly Trp Leu Ser met Arg Glu Trp Asp Asn Phe Leu

565   GTA CTG TAC TAT GAA GAC ATA AAA AAG GAT ACA AAG GGA ACC ATA AAG AAG ATC TGT GAC
181   Val Leu Tyr Tyr Glu Asp Ile Lys Lys Asp Thr Lys Gly Thr Ile Lys Lys Ile Cys Asp

625   TTC CTA GGG AAA AAT TTA GGG CCA GAT GAG CTG GAT CTC GTC CTC AAG TAC AGC TCT TTC
201   Phe Leu Gly Lys Asn Leu Gly pro Asp Glu Leu Asp Leu Val Leu Lys Tyr Ser Ser Phe

685   CAA GCC ATG AAA GAA AAC AAC ATG TCC AAT TTT AGT CTC ATT AAG GAA GAT CAG GTT ACT
221   Gln Ala Met Lys Glu Asn Asn Met Ser Asn Phe Ser Leu Ile Lys Glu Asp Gln Val Thr
                                ♦           ♦
745   AAT GGC TTG AAG CTC ATG AGA AAA GGC ACA ATT GGG GAC TGG AAG AAT CAC TTC ACA GTA
241   Asn Gly Leu Lys Leu Met Arg Lys Gly Thr Ile Gly Asp Trp Lys Asn His Phe Thr Val

805   GCC CAA GCT GAA GCC TTC GAT AAA GTT TTC CAG GAG AAA ATG GCT GGA TTC CCC CCA GGG
261   Ala Gln Ala Glu Ala Phe Asp Lys Val Phe Gln Glu Lys Met Ala Gly Phe Pro Pro Gly

865   ATT TTC CCA TGG GAA TAA TTTTCAAAGCTTTTGAATATTATATGAACACTGATGTTTATGTTCCTGTTGTTGT
281   Ile Phe Pro Trp Glu ***

938   GTATCTTGAATACCTAGATGTGGTCATAGAATAAAACCTGTTGTGGAAAAAAAAAAAAAAAAAA
```

Fig. 1. Nucleotide and deduced amino acid sequences of mouse ST_{a1} cDNA. Predicted asparagine *N*-linked glycosylation sites are indicated by filled diamonds. Stop codon is designated by ***. Consensus polyadenylation signal is denoted by boldface italic.

its deduced amino acid sequence. The cDNA contains an 855-nucleotide open reading frame (ORF) beginning at nucleotide 25 and encoding a 285-amino acid polypeptide (M_r, 33.2 kDa). There is no signal peptide insertion sequence or putative transmembrane hydrophobic domain indicative of a cytosolic protein. The translocation stop codon, *TAA*, is located 5′ of the consensus sequences for polyadenylation (*AATAAA*) and a short segment of poly$(A)^+$ tail is shown in Fig. 1.

Figure 2 shows the alignment of the deduced amino acid sequences for the family of STs obtained from different species. The mouse liver alcohol/hydroxysteroid ST (mST_{a1}) shares extensive amino acid sequence homology with the other ST family members from other species. This suggests an evolutionary conservation of important biological functions for this family of enzymes. The mST_{a1} shows the greatest homology with the rat senescence marker protein SMP-2, with 95 and 88% identities at the nucleotide and amino acid levels, respectively, (6). In addition, mST_{a1} shares 78, 78, and 64% homologies with $rST_{a\text{-}20}$ (4), rST_a (5), and hST_a, respectively, (12,13), and this finding suggests that it belongs to the alcohol/hydroxysteroid subfamily of sulfotransferases. Compared to the other subfamilies of sulfotransferases, such as phenol and estrogen, mST_{a1} shares 37, 37, 33, and 35% homologies with the rST_p (7), mST_{p1} (11), rST_e (8), and bST_e (10), respectively.

The alcohol/hydroxysteroid form of sulfotransferases had been shown to metabolize a variety of compounds containing primary or secondary aliphatic alcohols such as epiandrosterone, DHEA, β-estradiol, testosterone, *d*-aldosterone, and cortisol and drugs such as chloramphenicol, retinol (vitamin A), propranolol, and ephedrine (19). In addition, the alcohol/hydroxysteroid form of sulfotransferases had been implicated to play a critical role in the activation of certain carcinogens through O-sulfonations, e.g., 7-hydroxymethyl-12-methyl-benz[*a*]anthrancene (20) and 5-hydroxymethylchrysene (21), in rats. Questions remain as to which ST-a isoenzyme(s) catalyzes such reactions, and whether there are species differences in this metabolic pathway. The molecular cloning of mST_{a1} and other ST genes provides an impetus to address these issues.

```
m-ST-a1      1  M---MSDYNWFEG--IPFPAISYQREILEDIR--NKFVVKEEDLLILTYPKSGTNWLIEIVC
r-ST-smp     1  M---MSDYNWFEG--IPFPAISYEREILEDIR--NKFVVKEEDLLILTYPKSGTNWLNEIVC
r-ST-a       1  ----MPDYTWFEG--IPFPAFGIPKETLQNVC--NKFVVKEEDLILLTYPKSGTNWLIEIVC
r-ST-a20     1  ----MPDYTWFEG--IPFHAFGISKETLQNVC--NKFVVKDEDLILLAYPKSGTNWLIEIVC
h-ST-a       1  M---SDDFLWFEG--IAFPTMGFRSETLRKVR--DEFVIRDEDVIILTYPKSGTNWLAEILC
m-ST-p1      1  MAQNPSNMEPLRKPLVPVKGIPLIKYFAETMEQLQNFTAWPDDVLISTYPYSGTTWMSEIMD
r-ST-p       1  -------MEFSRPPLVHVKGIPLIKYPAETIGPLQNFTAWPDDLLISTYPKSGTTWMSEILD
r-ST-e       1  M---ETSMPEYYDVFGDFHGFLMDKRFTKYWEDVETFLARPDDLLIVTYPKSGSTWISEIVD
b-ST-e       1  M---SSSKPSFSDYFGKLGGIPMYKKFIEQFHNVEEFEARPDDLVIVTYPKSGTTWLSEIIC

            56  LIQTKGDPKWIQTVPIWNRSPWIE---TDI--GYSALINKEGPRLITSHLPIHLFSKSFFSS
            56  LIQTKGDPKWIQSCPFGTVYP-DE---IEW--IFRN--NHGGPRLITSHLPIHLFSKSFFSS
            55  LIQTKGDPKWIQSVTIWDRSPWIE---TDL--GYDMLIKKKGPRLITSHLPMHLFSKSLFSS
            55  LIQTKGDPKWIQSVTIWDRSPWIE---TDV--GYDILIKKKGPRLMTSHLPMHLFSKSLFSS
            56  LMHSKGDAKWIQSVPIWERSPWVE---SEI--GYTALSETESPRLFSSHLPIQLFPKSFFSS
            63  MIYQGGKLDKCGRAPVYARIPFLEFSCPGVPPGLETLKETPAPRIIKTHLPLSLLPQSLLDQ
            56  MIYQGGKLEKCGRAPIYARVPFLEFKCPGVPSGLETLEETPAPRLLKTHLPLSLLPQSLLDQ
            60  MIYKEGDVEKCKEDALFNRIPDLECRNEDLINGIKQLKEKESPRIVKTHLPAKLLPASFWEK
            60  MIYNNGDVEKCKEDVIFNRVPYLECSTEHVMKGVKQLNEMASPRIVKSHLPVKLLPVSFWEK

           113  KAKAIYLVRNPRDILVSGYFFWGNTNLVKNPGSLGTYFEWFLKGNVLFGSWFEHVRGWLSMR
           110  KAKAIYLMRNPRDILVSGYFFWGNTNLVKNPGSLGTYFEWFLQGNVLFGSWFEHVRGWLSMR
           112  KAKVIYLIRNPRDVLVSGYYFWGKTTLAKKPDSLGTYVEWFLKGYVPYGSWFEHIRAWLSMR
           112  KAKVIYLVRNPRDVLVSGYYFWGNSTLAKKPDSLGTYVEWFLKGNVLYGSWFEHIRAWLSMQ
           113  KAKVIYLMRNPRDVLVSGYFFWKNMKFIKKPKSWEEYFEWFCQGTVVYGSWFDHIHGWMPMR
           125  KIKVIYVARNAKDVVVSYYNFYKMAKLHPDPGTWESFLENFMDGKVSYGSWYQHVKEWWELR
           118  KVKVIYIARNAKDVVVSYYNFYNMAKLHPDPGTWDSFLENFMDGEVSYGSWYQHVKEWWELR
           122  NCKIIYLCRNAKDVVVSYYYFFLIMKSYPNPKSFSEFVEKFMEGQVPYGSWYDHVKSWWEKS
           122  NCKIIYLSRNAKDVVVSYYFLILMVTAIPDPDSFQDFVEKFMDGEVPYGSWFEHTKSWWEKS

           175  EWDNFLVLYYEDIKKDTKGTIKKICDFLGKNLGPDELDLVLKYSSFQAMKENNMSNFSLIKE
           172  EWDNFLVLYYEDMKKDTMGTIKKICDFLGKNLGPDELDLLLKYSSFQAMKENNMSNYSLIKE
           174  ELDNFLLLYYEDMKKDTMGTIKKICDFLGKKLEPDELDLVLKYSSFQVMKENNMSNYNLMEK
           174  EWDNFLLLYYEDMKKDTMGTIKKICDFLGKKLEPDELDLVLKYSSFQVMKENDMSNYSLLMK
           175  EEKNFLLLSYEELKQDTGRTIEKICQFLGKTLEPEELNLILKNSSFQTMKENKMSNYSLLSV
           187  RTHPVLYLFYEDMKENPKREIKKILEFLGRSLPEETVDLIVHHTSFKKMKENPMANYTTIPT
           180  HTHPVLYLFYEDIKENPKREIKKILEFLGRSLPEETVDSIVHHTSFKKMKENCMTNYTTIPT
           184  KNSRVLFMFYEDMKEDIRREVVKLIEFLERDPSAELVDRIIQHTSFQEMKNNPCTNYSMLPE
           184  KNPQVLFLFYEDMKENIRKEVMKLLEFLGRKASDELVDKIIKHTSFQEMKNNPSTNYTTLPD

           237  DQV-TNGLKLMRKGTIGDWKNHFTVAQAEAFDKVFQEKMAGFPPGIFPWE
           234  DPI-LTGLKLMRKGTTGDWKNHFTVAQAEAFDKVFQEFMAGFPPGMFPWE
           238  ELI-LPGFTFMRNGTTGDWKNHFTVAQAEAFDKVFQEKMAGFPPGMFPWD
           236  KSI-FTGTGLMRKGTVGDWKNHFTVSQAEAFDKVFQEKMAGFPPGMFPWE
           239  DYV-VDKAQLLRKGVSGDWKNHFTVAQAEDFDKLFQEKMADLPRELFPWE
           251  EVMDHTIYPFMRKGTIGDWKNTFTVAQSEHFDAHYAKLMTGCDFTFRCQI
           244  EIMDHNVSPFMRKGTTGDWKNTFTVAQNERFDAHYAKTMTDCDFKFRCEL
           248  TMIDLKVSPFMRKGIVGDWKNHFPEALRERFEEHYQQQMKDCPVKFRAEL
           248  EVMNQKVSPFMRKGDVGDWKNHFTVALNEKFDMHYEQQMKGSTLKFRTKI
```

Fig. 2. Alignment of the deduced amino acid sequences of mouse ST_{a1}, rat ST_{smp} (6), rat ST_a (5), rat ST_{a20} (4), human ST_a (12,13), mouse ST_{p1} (11), rat ST_p (7), rat ST_e (8), and bovine ST_e (10).

REFERENCES

1. G. J. Mulder and W. B. Jakoby. Sulfation. In G. J. Mulder (ed.), *Conjugation Reactions in Drug Metabolism,* Taylor & Francis, London, 1990, pp. 107–162.
2. R. M. Weinshilboum. Phenol sulfotransferase in humans: Properties, regulation, and function. *Fed. Proc.* 45:2223–2228 (1986).
3. T. N. Wight and R. P. Mecham. *Biology of Proteoglycans,* Academic Press, Orlando, FL, 1987.
4. K. Ogura, J. Kajita, H. Narihata, T. Watabel, S. Ozawa, K. Nagata, Y. Yamazoe, and R. Kato. Cloning and sequence analysis of a rat liver cDNA encoding hydroxysteroid sulfotransferase. *Biochem. Biophys. Res. Commun.* **165**:168–174 (1989).
5. K. Ogura, J. Kajita, H. Narihata, T. Watabel, S. Ozawa, K. Nagata, Y. Yamazoe, and R. Kato. cDNA cloning of the hydroxysteroid sulfotransferase STa sharing a strong homology in amino acid sequence with the senescence marker protein SMP-2 in rat livers. *Biochem. Biophys. Res. Commun.* **166**:1494–1500 (1990).
6. B. Chatterjee, D. Majumdar, O. Ozbilen, C. V. R. Murty, and A. K. Roy. Molecular cloning and characterization of cDNA for androgen-repressible rat liver protein, SMP-2. *J. Biol. Chem.* **262**:822–825 (1987).
7. S. Ozawa, K. Nagata, D. Gong, Y. Yamazoe, and R. Kato. Nucleotide sequence of a full-length cDNA (PST-1) for aryl sulfotransferase from rat liver. *Nucl. Acids Res.* **18**:4001 (1990).
8. W. F. Demyan, C. S. Song, D. S. Kim, S. Her, W. Gallwitz, T. R. Rao, M. Slomczynska, B. Chatterjee, and A. K. Roy. Estrogen sulfotransferase of the rat liver: Complementary DNA cloning and age- and sex-specific regulation of messenger RNA. *Mol. Endocrinol.* **6**:589–597 (1992).
9. Y. Hashimoto, A. Orellana, G. Gil, and C. B. Hirschberg. Molecular cloning and expression of rat liver *N*-heparan sulfate sulfotransferase. *J. Biol. Chem.* **267**:15744–15750 (1992).
10. A. R. Nash, W. K. Glenn, S. S. Moore, J. Kerr, A. R. Thompson, and E. O. P. Thompson. Oestrogen sulfotransferase: Molecular cloning and sequencing of cDNA for the bovine placental enzymes. *Aust. J. Biol. Sci.* **41**:507–516 (1988).
11. A.-N. T. Kong, M. Ma, D. Tao, and L. Yang. Molecular cloning of cDNA encoding the phenol/aryl form of sulfotransferase (mST_{p1}) from mouse liver. *Biochim. Biophys. Acta* **1171**:315–318 (1993).
12. D. M. Otterness, E. D. Wieben, T. C. Wood, W. G. Watson, B. J. Madden, D. J. McCormick, and R. M. Weinshilboum. Human liver dehydroepiandrosterone sulfotransferase: Molec-

ular cloning and expression of cDNA. *Mol. Pharmacol.* **41**:865–872 (1992).

13. A.-N. T. Kong, L. Yang, M. Ma, D. Tao, and T. D. Bjornsson. Molecular cloning of the alcohol/hydroxysteroid form (hST_a) of sulfotransferase from human liver. *Biochem. Biophys. Res. Commun.* **187**:448–454 (1992).
14. G. J. Mulder. *Sulfation of Drugs and Related Compound,* CRC Press, Boca Raton, FL, 1981.
15. R. W. Eastabrook, L. Milewich, and R. A. Prough. Cytochrome P-450s as toxicogenic catalysts: The influence of dehydroepiandrosterone. In L. Ernster, H. Esumi, Y. Fujii, H. V. Gelboin, R. Kato, and T. Sugimura (eds.), *Xenobiotics and Cancer,* Taylor & Francis Ltd., Bristol, PA, 1991, pp. 33–44.
16. F. M. Ausubel, R. Brent, R. F. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and K. Struhl (eds.), *Current Protocols in Molecular Biology,* Green Associates and Wiley Interscience, New York, 1990.
17. A. P. Feinberg and B. Vogelstein. A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. *Anal. Biochem.* **132**:6–13 (1983).
18. F. Sanger, S. Nicklen, and A. R. Coulson. DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci. USA* **74**:5463–5467 (1977).
19. E. S. Lyon and W. B. Jakoby. The identity of alcohol sulfotransferases with hydroxysteroid sulfotransferases. *Arch. Biochem. Biophys.* **202**:474–481 (1980).
20. T. Watabe, T. Ishizuka, M. Isobe, and N. Ozawa. A 7-hydroxymethyl sulfate ester as an active metabolite of 7,12-dimethylben[a]anthracene. *Science* **215**:403–405 (1982).
21. H. Okuda, A. Hiratsuka, H. Nojima, and T. Watabe. A hydroxymethyl sulphate ester as an active metabolite of the carcinogen, 5-hydroxymethylchrysene. *Biochem. Pharmacol.* **35**:535–538 (1986).