# Characterization of bovine tracheobronchial phenol sulphotransferase cDNA and detection of mRNA regulation by cortisol

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Phenol sulphotransferases esterify both endogenous and foreign hydroxylated aromatic compounds with sulphate. Since these enzymes participate in both hormone and drug metabolism, elucidating their regulation at both the enzymic and molecular levels may provide new understanding in several metabolic pathways. The primary structure of a bovine phenol sulphotransferase has been determined by isolation of the corresponding cDNA. Two partial bovine cDNAs were first isolated by probing a tracheal epithelial cell λgt11 cDNA library with a rat phenol sulphotransferase cDNA. These clones provided the sequences of the 5′ and 3′ ends of the predicted coding region. A contiguous cDNA was subsequently isolated by PCR using 5′ and 3′ oligonucleotide primers and the cDNA library as the template. The sequence of the resulting approx. 1 kbp cDNA predicted an amino acid sequence that included sequences determined for several tryptic peptides of the purified protein. Antiserum directed to a synthetic N-terminal peptide predicted by the cDNA sequence showed reactivity with the purified enzyme. High-level Trc-promoter-driven expression of the recombinant bovine enzyme was achieved in *Escherichia coli*. The bovine cDNA was used to determine relative steady-state levels of phenol sulphotransferase transcripts in bovine lung tissues; distal lung parenchymal RNA levels were 6–10-fold greater than those in tracheobronchial epithelium. Using a bronchial epithelial cell culture model, however, cortisol was observed to increase mRNA levels by 5-fold in both a dose- and time-dependent manner; this corresponds to previously reported glucocorticoid stimulation of phenol sulphotransferase activity in this system [Beckmann, Illig and Bartzatt (1994) J. Cell Physiol. **160**, 603–610].

## INTRODUCTION

Sulphate has diverse functions in virtually all biological systems. In some microbial organisms sulphate is reduced to sulphite or hydrogen sulphide, thus being an electron acceptor for oxidative pathways. The utilization of sulphate for biosynthetic pathways often requires covalent attachment to ATP to form adenosine 5′-phosphosulphate, which is subsequently phosphorylated to form 3′-phosphoadenosine 5′-phosphosulphate (PAPS). In higher eukaryotes, PAPS is utilized by a wide variety of enzymes, generically known as sulphotransferases [1]. These enzymes catalyse the sulphonation of diverse acceptor substrates ranging from small endogenous or foreign compounds, such as catecholamines or phenol, to large macromolecules such as heparan [2]. This paper focuses on the former, specifically a phenol sulphotransferase (PST; EC 2.8.2.1) derived from bovine lung.

Sulphonation of many drugs is an important route of metabolism [3,4] and may also play an important role in the detoxification of carcinogenic compounds [5–7]. Sulphotransferases, however, can convert some xenobiotics into ultimate carcinogens [8,9]. Regulation of either the activities or the expression of these enzymes is therefore under current investigation [10–13], and most recently this is being extended by molecular biological methods [14,15]. Although the liver is the organ generally accepted as the major site for sulphate conjugation [16,17], sulphotransferases are also present in several other locations [18–20] including lung [21]. Indeed, inhaled xenobiotics such as benzene can be detoxified predominately by sulphation [22], and there are some data that indicate that this conjugation could occur within the lung *in vivo* [23]. Using human bronchial explant cultures, Gibby and Cohen [24] demonstrated that sulphonation predominates as a conjugative pathway at low (20 μM) naphthol concentrations. This was largely in agreement with Autrup *et al.* [25], who compared the metabolism of benzo[*a*]pyrene by human and bovine bronchial tissues; a quantitative similarity between these species was noted.

Within the bovine respiratory tract epithelium, the level of expression of PST is dependent on the location and cell type [26]; the greatest enzyme activity is seen in distal lung tissue, and this correlates with the greatest immunopositivity in non-ciliated bronchiolar epithelial cells. Although the level of PST may be relatively low in the tracheobronchial epithelium, this tissue is complex both cellularly and with respect to possible neoplastic states of differentiation to non-ciliated epithelial cell types. Therefore we have been testing the hypothesis that PST expression in tracheobronchial epithelium can be regulated, using a bovine bronchial epithelial cell culture model system [27]. In a previous report, we have shown that cortisol stimulates expression of PST activity and protein levels in this model [13]. Here we report on the isolation and characterization of a bovine PST cDNA, we show that transcript levels vary along the respiratory tract epithelium as expected, and we demonstrate that cortisol increases levels of cognate transcript levels in the cultured airway epithelial cells in a concentration- and time-dependent manner.

## MATERIALS AND METHODS

### Materials and reagents

Amplitaq DNA polymerase, nucleotides and buffers were from Perkin-Elmer/Cetus. Restriction endonucleases, Vent DNA

Abbreviations used: PAPS, 3′-phosphoadenosine 5′-phosphosulphate; 1 × SSPE, 0.18 M NaCl/10 mM sodium phosphate/1 mM EDTA, pH 7.4; PST, phenol sulphotransferase; TL-PST, thermolabile PST; TS-PST, thermostable PST; IPTG, isopropyl β-D-thiogalactopyranoside.

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polymerase and cycle sequencing kit, T4 polynucleotide kinase, T4 DNA ligase and Klenow fragment of DNA polymerase were from New England Biolabs (Beverly, MA, U.S.A.). For some sequencing reactions, the Sequitherm cycle sequencing kit from Epicentre Technologies (Madison, WI, U.S.A.) was employed. $^{32}$P-labelled nucleotides were from Amersham (Arlington Heights, IL, U.S.A.). Buffers and salts were analytical reagent grade. The rat minoxidil sulphotransferase cDNA [28], which is identical to rat PST-1 [29], was kindly provided by Dr. Charles Falany (University of Alabama, Birmingham, AL, U.S.A.). The plasmids pT7T3-18U and pTrc-99A for bacterial expression were obtained from Pharmacia (Piscataway, NJ, U.S.A.). Reagents and procedures for assay of PST activities were as previously described [13,30,31].

### Purification of bovine lung PST

Portions (approx. 100 g) of fresh bovine lungs obtained at a local slaughterhouse were chilled on ice prior to homogenization in a Waring blender in 1 litre of 20 mM Hepes, 20% (w/v) sucrose, 5 mM 2-mercaptoethanol, 1 mM EDTA, pH 7.4 (Buffer A), including 1 mM PMSF. Homogenates were centrifuged for 1 h at 45000 *g* to obtain the clear pink crude extract supernatants. Stock 5 M NaCl was added to give a final salt concentration of 0.4 M and the extract was applied directly to an Affigel Blue (Bio-Rad, Richmond, CA, U.S.A.) column (20 cm × 5 cm) in Buffer A. After a 1.6 litre wash with Buffer A containing 0.4 M NaCl, bound proteins were eluted with Buffer A plus 2 M NaCl. Fractions containing greatest activity [30,31] were pooled (approx. 200 ml), and then diluted with 50 ml of 20 mM Tris/Cl, pH 7.4, to dilute the sucrose. Solid ammonium sulphate was added slowly with gentle stirring on ice to 32.6 g/100 ml (55% saturation), followed by 20000 *g* centrifugation. The protein pellets were dissolved in 50 ml of Buffer A and exhaustively dialysed against three changes of 500 ml of Buffer A. Precipitated protein was removed by centrifugation and the sample was applied to DEAE-cellulose (DE-52; Whatman) equilibrated in Buffer A (2.5 cm × 18 cm column). The loaded column was washed with 200 ml of Buffer A, followed by elution with a 1.8 litre gradient from 0 to 0.4 M NaCl in Buffer A. Fractions containing peak PST activity were pooled and concentrated to 3 ml using a stirred pressurized Amicon apparatus. Insoluble protein was removed by centrifugation, and the clear supernatant was passed over a column (2.5 cm × 50 cm) of Sephadex G-100 equilibrated in Buffer A. Peak activity fractions that eluted immediately after the void volume were pooled and concentrated by centrifugation (Centriprep units; Millipore) to 3 ml, and applied to 1 ml of ATP–agarose in Buffer A. (Note that although we report here the use of ATP–agarose, which was the Sigma product used at the time, subsequent lots of this product have not been useful and we have been able to use instead ADP–agarose.) The loaded affinity resin was washed with 10 ml of Buffer A, followed by elution of the PST with the buffer containing 40 $\mu$M PAPS (Sigma). Aliquots from 1 ml fractions were subjected to analytical SDS/PAGE [32] and enzyme assay prior to pooling and concentration. A purification table summarizing this procedure is presented (Table 1).

### Tryptic peptide isolation and sequencing

Purified PST (60 $\mu$g in three lanes) was subjected to PAGE [32] followed by electrotransfer to poly(vinylidene difluoride) membrane (Immobilon; Milipore) in 10 mM CAPS/10% (v/v) methanol, pH 11 [33]. Bands of 32 kDa visualized without staining were excised, reductively alkylated with iodoacetate, washed in water and subjected to *in situ* trypsinization [34]. Fragments eluted with 70% (v/v) propan-2-ol were HPLC-purified by standard methods and sequenced by automated Edman degradation using an ABI 477A/120A instrument.

### RNA isolation and analyses

Total RNA was purified either from fresh bovine lung tissues or from cultured bovine bronchial epithelial cells according to a previous method [35]. Samples were quantified by absorbance using Warburg coefficients as a component of a Beckman DU-62 spectrophotometer. RNA samples of 10–20 $\mu$g were subjected to electrophoresis through 1% agarose gels containing formaldehyde [36] and ethidium bromide (1 $\mu$g/ml), capillary-blotted directly to nylon membranes (Magnagraph; MSI) using 20 × SSPE [36], and covalently linked to the membrane by ultraviolet illumination (Stratalinker; Stratagene). These Northern blots were subsequently blocked for at least 1 h in 2 × SSPE, 5 × Denhardt's solution, 0.5% (w/v) SDS at 42 °C prior to hybridization to the indicated probes in 2 × SSPE, 1 × Denhardt's, 0.2% SDS, 5% (w/v) dextran sulphate, 50% (v/v) formamide at 42 °C for 16–48 h with gentle agitation. Probes were prepared by random hexamer priming [37] using [$^{32}$P]dCTP (20 $\mu$Ci/hybridization) and heat-denatured for 5–10 min at 95 °C just prior to dilution into hybridization fluid (10–15 ml). Post-hybridization washing conditions were varied between probes as indicated in the Figure legends.

Relative mRNA levels were determined by scanning densitometry using a Hoefer GS-300 instrument in transmittance mode. Integration was accomplished by an interfaced Macintosh SE computer. Two autoradiographic exposures were measured in order to validate the quantifications.

### cDNA library screening and clone analysis

Total RNA was extracted [35] from three freshly dissected bovine tracheal epithelial mucosae. The pooled RNA was submitted for custom cDNA library synthesis using a combination of oligo(dT) and random primed reverse transcription, using bacteriophage λgt11 as the host vector (Clontech, Palo Alto, CA, U.S.A.). The library, which consisted of $1.5 \times 10^6$ independent recombinants, was plated at approx. 100000 plaques per 127 mm × 178 mm dish, and from 10 such dishes duplicate lifts with nitrocellulose membranes were taken. These lifts were blocked and hybridized (see above) using the rat minoxidil sulphotransferase cDNA as a heterologous probe; hybridized lifts were washed four times in 0.1 × SSPE, 0.1% SDS at 23 °C, since preliminary experiments using this cDNA to probe bovine lung Northern blots indicated that higher-stringency washes released the probe (results not shown). Positive plaques were subjected to two more rounds of plating, lifting, and hybridization, at which point purity was demonstrated by positive hybridization of 100% of the plaques.

Initial clone characterization was accomplished by PCR amplification of the cDNA inserts using flanking λ oligonucleotide primers followed by partial sequencing. After verification, cDNA inserts liberated from purified λ DNA by *Eco*RI digestion were subcloned into plasmid pT7T3-18U. To complete the sequencing of these isolates, internal oligonucleotide primers were synthesized (Integrated DNA Technologies, Coralville, IA, U.S.A.) and used for thermal cycle sequencing.

To obtain a contiguous bovine PST cDNA, PCRs were conducted. Converging 5′ upstream (5′-GGAATTCCCTGAGAAGATAGCATGG-3′) and 3′ downstream (5′-GGAATTGGGTCTGTTTCACATGCC-3′) primers were designed based on the sequences of clones 2 and 1 respectively. The bovine tracheal cDNA library (above) was used as the template at 4 $\mu$l per 50 $\mu$l

reaction, or approx. $3 \times 10^7$ clones per reaction. Attempts using Amplitaq DNA polymerase were initially unsuccessful; however, the less error-prone Vent DNA polymerase amplified an approx. 1 kbp product which was characterized in detail. This 1 kbp band was excised from low-melting agarose and used for subsequent re-amplifications of subfragments that were used in cycle sequencing reactions. For generation of the 1 kbp DNA fragment, cycling parameters were 90 s at 94 °C, 90 s at 45 °C and 90 s at 72 °C. Subfragment generation thermocycling parameters were shortened to 60 s incubations at these temperatures. In addition to directly sequencing such amplification products, which avoids potential errors compared with individual cloned products, two cloned PCR products used for expression (see below) were also sequenced using an Applied Biosystems (Foster City, CA, U.S.A.) Model 373 sequences and protocols supplied by the manufacturer.

Analyses of nucleotide and amino acid sequences were accomplished through the use of the Apple Macintosh computer program GeneJockey (Biosoft, Cambridge, U.K.). This package includes subroutines for matrix alignment, PCR primer design and hydropathy analysis. For this last method, the Kyte–Doolittle algorithm with a 7-amino-acid window width was employed.

### Expression of bovine PST in *E. coli*

Attempts to clone the Vent-polymerase-amplified cDNA were unsuccessful. Therefore converging primers were re-designed to include a 5′ *Nco*I restriction site at the ATG initiation codon (5′CCTGAGAAGATACCATGGAACTG), and a 3′ *Xba*I site (5′-GGTCTAGAGGACCACTCACAGC) just past the termination codon of the predicted open reading frame (see Figure 2). Amplitaq DNA polymerase amplified a contiguous cDNA from the tracheal cDNA library (see above) using optimized cycling parameters of 1 min at 94 °C, 1 min at 60 °C and 1 min at 72 °C. PCR products were ligated into the TA cloning kit vector pCR-II (In Vitrogen, San Diego, CA, U.S.A.), from which several clones were obtained that contained an approx. 900 bp insert liberated by digestion with *Nco*I plus *Xba*I. Fragments purified by electrophoresis through low-melting-point agarose (GTG grade; FMC, Rockland, ME, U.S.A.) were directly ligated to digested pTrc-99A and transformed into *E. coli* JM101 cells. Corresponding expression vector subclones from the initial pCRII isolates were thus obtained. Preliminary experiments demonstrated expression of an SDS-solubilized 32 kDa protein in such transformants after overnight culture at 37 °C in the presence of 1 mM isopropyl β-D-thiogalactopyranoside (IPTG). For preparation of active enzyme extracts, 75 ml cultures of LB medium plus 100 μg/ml ampicillin and 2 mM IPTG were inoculated with either pTrc-99A- or pTrc-PST-14-transformed JM101 cells and grown overnight. Cells collected by centrifugation were resuspended in 7.5 ml of cold Buffer A and sonicated using three 30 s bursts with intermittent cooling of the microtip in cold buffer. Cytosolic extracts obtained after a 1 h centrifugation at 45000 *g* were stored frozen at −80 °C for up to 1 week prior to assays for protein, PST activities or immunoblot analyses [26].

### Peptide syntheses and immunochemical methods

We have previously reported on the preparation of mouse anti-(bovine PST) using the purified enzyme as immunogen [26]. In order to test the expression of the N-terminal region predicted by the cDNA sequence, a synthetic peptide (MELIQDTSRPPA-KYC; amidated C-terminus) was conjugated chemically (Imject kit; Pierce, Rockford, IL, U.S.A.) via the C-terminal cysteine thiol to keyhole limpet haemocyanin and injected into mice. Primary injections utilized 100 μg of conjugate and boosts of 50 μg were injected after each of two 3 week intervals. Enzyme-linked immunosorbant assays, directed against the above peptide conjugated to BSA and also to purified PST, demonstrated an anti-peptide immune response (results not shown).

Immunoblot analyses were performed by standard methods [38] using Nitroplus membrane (Micron Separations, Inc.). Blots were prestained with Ponceau S to ensure transfer. Blocking was achieved using 20 mM Tris/HCl, 100 mM NaCl, pH 8 (TBS) plus 0.2% (v/v) Tween 20 (Fisher) for 1–2 h, followed by probing with 1:2500 dilutions of sera in this TBST solution. After several TBS washes, blots were incubated with diluted peroxidase-conjugated anti-mouse (ICN) in TBST and washed extensively with TBS, followed by chemiluminescent detection (ECL kit; Amersham).

### Cell culture

Media, supplements and primary bovine bronchial epithelial cells were prepared as previously described [27]. Cells were resuspended in a 1:1 (v/v) mixture of RPMI 1640 and LHC Basal medium (Biofluids, Rockville, MD, U.S.A.) supplemented with calcium stock, trace elements, Stocks 4 and 11, Pen/Strep and Fungizone (LHC-D) plus bovine pituitary extract (100 μg of protein/ml) and insulin (5 μg/ml). After overnight attachment to plastic tissue culture dishes (35 mm) that were pretreated with 30 μg of type I collagen (Vitrogen; Collagen Corp., Palo Alto, CA, U.S.A.), unattached cells were removed by two or three washes. Cells were then fed with LHC-D/RPMI supplemented with insulin. After an additional 3 days, cultures were stimulated for various times and doses of cortisol, as indicated in the Figure legends.

## RESULTS

### Purification of bovine PST and peptide sequence analyses

We have previously reported that greatest PST enzyme activity was detected in distal bovine airway tissue. Lung parenchyma was therefore used for enzyme purifications (Table 1). The final enzyme preparation was purified over 1300-fold relative to the first centrifuged extract, and this is reflected in the high degree of purity indicated by PAGE in the presence of SDS (Figure 1). The final specific activity of 4600 nmol/min per mg, when assayed under optimal conditions of pH 5.5 with 20 μM 2-naphthol as substrate [26], was comparable with several reported values for

**Table 1 Summary of purification of bovine lung PST**

Protein content was determined relative to BSA using a Coomassie G-250 dye binding assay (Bio-Rad). One unit of activity catalyses the formation of 1 nmol of 2-naphthyl sulphate/min at 23 °C, pH 5.5. These assays were conducted using 20 μM 2-naphthol [30,31].

| Sample | Protein (mg) | Activity (units) | Specific activity (units/mg) | Yield (%) |
|---|---|---|---|---|
| Cytosol | 19304 | 65970 | 3.4 | 100 |
| Affigel Blue | 412 | 76000 | 184 | 115 |
| Ammonium sulphate | 460 | 66435 | 144 | 101 |
| DE-52 | 76.8 | 6700 | 87* | 10 |
| Sephadex G-100 | 10.0 | 7500 | 393 | 11 |
| ATP–agarose | 1.77 | 8100 | 4600 | 12 |

* Value may be low due to NaCl present in the sample as a result of column gradient elution. The bovine lung enzyme is inhibited by millimolar concentrations of NaCl (not shown).

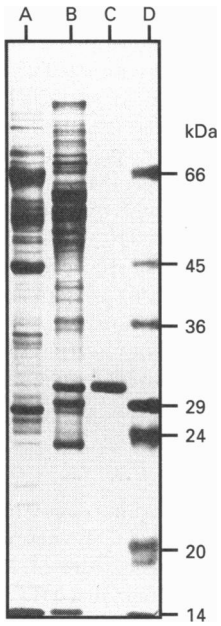


**Figure 1 Analysis of purification of bovine lung PST by PAGE**

Samples were denatured with SDS/2-mercaptoethanol prior to application to a 10% discontinuous gel according to Laemmli [32]. Lanes and loads were: A, alveolar cytosolic extract, 25 $\mu$g; B, pooled activity peak from DE-52, 25 $\mu$g; C, final enzyme eluted from ATP–agarose, 4 $\mu$g; D, Mark VII markers (Sigma; molecular mass values shown). The minimal staining sensitivity was about 0.1 $\mu$g; therefore the final preparation contains $< 0.1/4 = 2.5\%$ of any single impurity.

**Table 2 Amino acid sequences of bovine lung PST peptides**

The amino acid sequences were predicted from the following cDNA sequence reports: rat PST-1 [28,29]; bovine OST (oestrogen sulphotransferase) [41]; human P- (or thermostable) PST [40].

| | Identity (%) with: | | |
|---|---|---|---|
| Bovine PST sequence | Rat PST-1 | Bovine OST | Human P-PST |
| **26**: Ile-Leu-Glu-Phe-Ile-Gly-Arg | 85.7 | 71.4 | 85.7 |
| **27**: Ser-Leu-Pro-Glu-Glu-Thr-Val-Asp-His-Ile-Val-Gln-Arg | 76.9 | 30.8 | 76.9 |
| **28**: Asp-Val-Ala-Val-Ser-Tyr-Tyr-His-Phe-Tyr-Arg | 72.7 | 54.5 | 90.9 |
| **51**: Lys-Asn-Pro-Met-Thr-Asn-Tyr-Ser-Thr-Ile-Pro-Thr-Ala-Val-Met | 73.3 | 60.0 | 66.7 |
| **57**: Val-Pro-Phe-Leu-Glu-Phe-Ser-Ala-Pro-Gly-Val-Pro-Thr-Gly-Val-Glu-Leu-Leu | 72.2 | 50.0 | 72.2 |
| **61**: Tyr-Phe-Ala-Glu-Ala-Leu-Gly-Pro-Leu-Glu-Ser-Phe-Glu-Ala-Xaa-Pro-Asp-Asp-Leu-Leu-Ile-Ser-Thr-Tyr-Pro | 76.0 | 56.0 | 88.0 |

purified rat liver PSTs. It is not clear why our reported specific activities are 1000-fold greater than previously reported for bovine lung PST [39], although differences in assay pH may account for this discrepancy.

The purified PST protein was digested with trypsin and several peptides were sequenced (Table 2). At the time these data were obtained, the only other PST sequence data available were for a rat liver enzyme [29]. Comparison of the bovine PST peptide sequences with the corresponding predicted rat peptides clearly indicated significant identity (73–86%; Table 2). Comparison with the more recently reported human thermostable P-PST [40] indicated even greater average identities of up to 91%. Much less overlap (31–60%) was observed with the corresponding predicted bovine oestrogen sulphotransferase peptides [41]. These results strengthened the notion that the purified protein was a member of the PST family and that it was distinct from bovine oestrogen sulphotransferase.

### Isolation of partial bovine PST cDNAs using a heterologous probe

Northern blots of bovine lung and rat liver RNAs were probed with the rat minoxidil sulphotransferase cDNA [28] under various hybridization conditions followed by various washing stringencies. Optimal conditions were thus determined for the detection of a bovine transcript of identical size compared with that extracted from rat liver (results now shown). Plaque lift hybridization screening of a bovine tracheal epithelial cDNA library (see the Materials and methods section) yielded an initial seven positive plaques, of which two clones survived through tertiary screening and purification. PCR amplification of the inserts revealed sizes of approx. 800 bp and 500 bp (results not shown), which was confirmed by excision of the inserts from the $\lambda$gt11 vector with *Eco*RI.

The nucleotide sequences of the two bovine tracheal cDNA clones were determined. The 3′ end of clone 1 shared identity with the 3′ end of the minoxidil (rat PST-1) sulphotransferase [28,29] (results not shown). Interestingly, the 5′ end of bovine clone 1 shared no identity with any known sulphotransferase cDNA. No internal *Eco*RI endonuclease site was observed in clone 1, reducing the likelihood of a chimeric cloning artifact during library construction. At this time, the 5′ end of clone 1 remains unidentified. The entire sequence of bovine tracheal clone 2 was identical with the 5′ end of the rat cDNA [28,29] (results not shown). The sequences of the two clones did not overlap, however, and it was therefore impossible to deduce the contiguous bovine PST cDNA sequence.

### Isolation and analysis of a contiguous bovine PST cDNA

The complete bovine PST cDNA was obtained from the tracheal epithelial cDNA library by PCR using converging primers synthesized based on the 5′ end of clone 2 and on the 3′ end of clone 1 respectively (see the Materials and methods section). Nested PCR primers derived from clone 1 and 2 sequences and Southern blot hybridization analysis with the rat minoxidil sulphotransferase cDNA indicated the authenticity of the approx. 1 kbp PCR product (results not shown). The complete nucleotide sequence analysis of the bovine PST cDNA is shown in Figure 2. The sequence of the initially isolated clone 1 overlapped the PCR amplification product beginning at base 528 (not shown). The upstream 275 bp of clone 1, however, exhibited no discernable overlap with the final PCR sequence as tested by both matrix homology and simple alignment searches (not shown). The sequence of the aforementioned clone 2 extended to base 459 (not shown). The amplified cDNA predicts an 882 bp open reading frame encoding a 34 kDa protein of 294 amino acid residues. There was complete agreement between this sequence and the sequences of the six tryptic peptides derived from the purified bovine lung PST. Within the coding region, the bovine cDNA shows 79%, 85.4% and 83.8% identity with the rat minoxidil sulphotransferase [28,29], the human thermostable P-PST [40] and the human thermolabile M-PST [42] cDNA sequences respectively. At the amino acid sequence level, the

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1                                   GAATTCGGCCACACCCCGGCGCCCCTGAGAAGATAGC

   Met Glu Leu Ile Gln Asp Thr Ser Arg Pro Pro Ala Lys Tyr Val Lys Gly   17
38 ATG GAA CTG ATC CAG GAT ACC TCC CGC CCT CCA GCA AAG TAT GTG AAG GGC

   Ile Pro Leu Ile Lys Tyr Phe Ala Glu Ala Leu Gly Pro Leu Glu Ser Phe   34
89 ATC CCT CTC ATC AAG TAC TTT GCA GAG GCC CTG GGG CCA CTG GAG AGC TTT

   Glu Ala Trp Pro Asp Asp Leu Leu Ile Ser Thr Tyr Pro Lys Ser Gly Thr   51
140 GAA GCC TGG CCT GAT GAC TTG CTC ATC AGC ACC TAC CCC AAG TCG GGC ACC

   Thr Trp Val Ser Glu Ile Leu Asp Leu Ile Tyr Gln Glu Gly Asp Leu Glu   68
191 ACC TGG GTA AGC GAG ATC CTG GAC CTG ATC TAC CAG GAG GGT GAC CTG GAG

   Lys Cys Gln Arg Ala Pro Val Phe Leu Arg Val Pro Phe Leu Glu Phe Ser   85
242 AAG TGT CAG AGA GCC CCT GTC TTC TTG CGG GTG CCC TTC CTT GAG TTC AGC

   Ala Pro Gly Val Pro Thr Gly Val Glu Leu Leu Lys Asp Thr Pro Ala Pro  102
293 GCC CCT GGG GTT CCC ACA GGT GTT GAG CTT CTG AAA GAT ACA CCA GCC CCA

   Arg Leu Leu Lys Thr His Leu Pro Leu Ala Leu Leu Pro Lys Thr Leu Leu  119
344 CGG CTC CTC AAG ACA CAC TTG CCT CTG GCC CTG CTT CCG AAG ACC CTG CTG

   Asp Gln Lys Val Lys Val Ile Tyr Ile Ala Arg Asn Ala Lys Asp Val Ala  136
395 GAT CAG AAA GTC AAG GTG ATC TAC ATC GCC CGC AAC GCC AAG GAT GTG GCC

   Val Ser Tyr Tyr His Phe Tyr Arg Met Ala Lys Val His Pro Asp Pro Gly  153
446 GTC TCC TAT TAC CAC TTC TAT CGC ATG GCC AAG GTG CAC CCT GAC CCT GGC

   Thr Trp Asp Ser Phe Leu Glu Lys Phe Met Ala Gly Glu Val Cys Tyr Gly  170
497 ACC TGG GAC AGC TTC CTG GAG AAG TTC ATG GCC GGG GAA GTG TGC TAC GGG

   Ser Trp Tyr Gln His Val Gln Glu Trp Trp Glu Leu Ser His Thr His Pro  187
548 TCC TGG TAC CAG CAC GTG CAG GAG TGG TGG GAG CTG AGT CAC ACC CAC CCT

   Val Leu Tyr Leu Phe Tyr Glu Asp Ile Lys Glu Asp Pro Lys Arg Glu Ile  204
599 GTT CTC TAC CTC TTC TAC GAG GAC ATA AAG GAG GAC CCC AAA AGG GAG ATT

   Gln Lys Ile Leu Glu Phe Ile Gly Arg Ser Leu Pro Glu Glu Thr Val Asp  221
650 CAG AAG ATC CTG GAG TTT ATA GGG CGC TCC CTG CCT GAG GAG ACT GTG GAT

   His Ile Val Gln Arg Thr Ser Phe Lys Glu Met Lys Lys Asn Pro Met Thr  238
701 CAC ATT GTC CAG CGC ACA TCT TTC AAA GAG ATG AAG AAG AAC CCC ATG ACC

   Asn Tyr Ser Thr Ile Pro Thr Ala Val Met Asp His Ser Ile Ser Ala Phe  255
752 AAC TAC AGC ACC ATA CCC ACT GCA GTC ATG GAC CAC AGC ATC TCT GCC TTC

   Met Arg Lys Gly Ile Thr Gly Asp Trp Lys Ser Thr Phe Thr Val Ala Gln  272
803 ATG AGG AAA GGC ATC ACT GGT GAT TGG AAA TCC ACC TTC ACT GTG GCC CAG

   Asn Glu Leu Phe Glu Ala His Tyr Ala Lys Lys Met Arg Ala Ala Thr Pro  289
854 AAT GAG CTC TTT GAA GCC CAC TAT GCT AAG AAG ATG CGG GCT GCA ACT CCG

   Leu Arg Trp Glu Leu Stop                                            294
905 CTT CGC TGG GAG CTG TGA GTGGTCCTCTGGAGGCATGTGAAACAGACCCACGGGAATTC
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**Figure 2 Nucleotide and predicted amino acid sequence of bovine PST**

The *italicized* 5′ and 3′ nucleotide sequences, which include terminal *Eco*RI sites, were donated by cDNA clones 2 and 1 respectively. The PCR-amplified contiguous cDNA sequence is in non-italicized type. The underlined amino acid sequences are those determined for tryptic peptides of the purified protein (Table 2).

predicted bovine PST has 77%, 83% and 79% identity with the rat minoxidil sulphotransferase, the human thermostable P-PST and the human thermolabile M-PST enzymes. Thus the bovine PST cDNA appears most closely related to the human P-PST. Comparison was also made with the purported bovine oestrogen sulphotransferase [41] sequences; at the DNA and protein levels, these sequences are 55% and 50% identical respectively.

### Predicted N-terminal peptide antisera bind to purified bovine PST

Attempts to determine the N-terminal amino acid sequence of purified bovine lung PST were unsuccessful, as reported with other sulphotransferases [42,43]. To test for the presence of the N-terminus as predicted by the cDNA sequence open reading frame (Figure 2), antisera to the synthetic peptide MELIQDT-SRPPAKYC conjugated to keyhole limpet haemocyanin were elicited in mice. Immunoblot analyses (Figure 3) demonstrated reactivity with the purified lung enzyme, indicating that the peptide was in fact present on the protein synthesized *in vivo*. Binding of the antiserum to non-denatured PST was also suggested by indirect enzyme-linked immunosorbant assays (results not shown), which indicated the availability of the N-terminus on the native enzyme. Tests to determine if the anti-sera inhibited PST activity were negative, suggesting that the N-terminus is not a crucial component of the catalytic domain. This is in contrast with anti-(whole PST) antiserum, which neutralized catalytic activity [26].

### Expression of the bovine PST cDNA in *E. coli*

Although the above results provide strong evidence for the identity of the isolated cDNA sequence, confirmation was

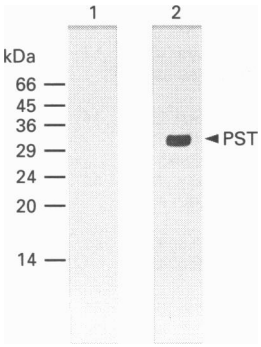


**Figure 3 Antiserum against the predicted N-terminus of bovine PST recognizes the purified protein**

Each lane contained 0.9 $\mu$g of purified enzyme blotted on to reinforced nitrocellulose (see the Materials and methods section). Lane 2 contains the anti-peptide antiserum. The control (lane 1) was probed with preimmune serum; both sera were diluted 1:2500. Detection was achieved by peroxidase-conjugated anti-mouse IgG and an ECL chemiluminiescence kit (Amersham).

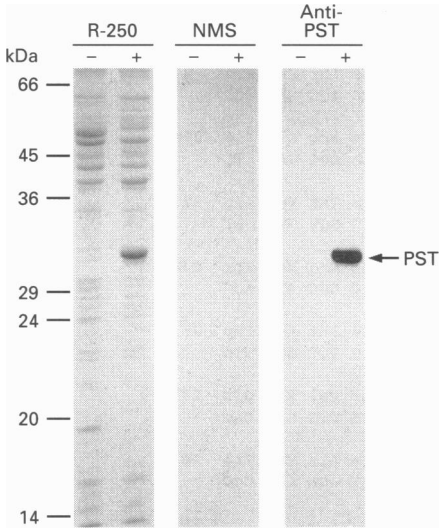


**Figure 4 Detection of recombinant bovine PST protein expression in *E coli***

JM101 cells were transformed (see the Materials and methods section) with either pTrc-99A vector (−) or pTrc-PST-14 (+) and subsequently grown overnight in the presence of 2 mM IPTG. Cytosolic extracts (see the Materials and methods section; 40 $\mu$g of protein per lane) were applied as shown to a 11% polyacrylamide gel and then blotted on to an Immobilon membrane. R-250, gel stained with Coomassie R-250; NMS, blot probed with normal mouse serum; anti-PST, blot probed with anti-(bovine PST). Detection was by chemiluminescence.

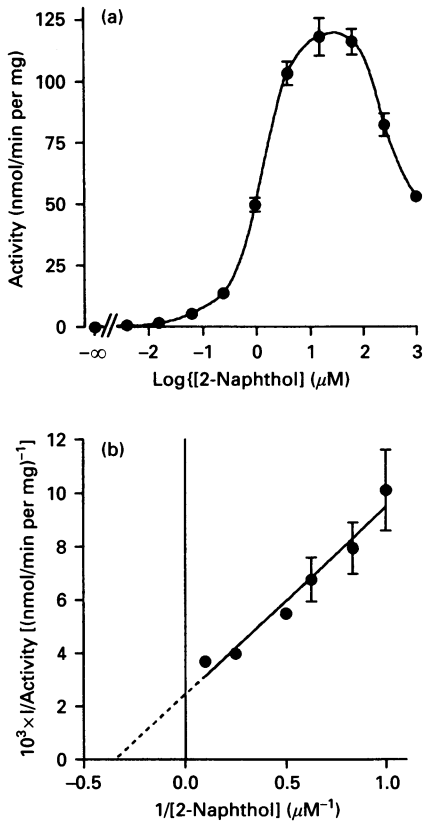


**Figure 5 Characterization of bovine PST activity expressed on *E. coli***

A cytosolic extract of JM101[pTrc-PST-14] (see Figure 4) was assayed at the indicated 2-naphthol concentration (see the Materials and methods section). The direct plot (**a**) suggests saturation kinetics followed by substrate inhibition. The reciprocal plot of a separate series of assays (**b**) indicates an apparent $K_m$ for 2-naphthol of 2.9 $\mu$M. The symbol size covers ± S.D. of triplicate assays unless shown otherwise.

achieved by inserting the cDNA into an *E. coli* expression vector (see the Materials and methods section). Whereas cells harbouring the pTrc-99A plasmid vector did not express immunodetectable PST protein (Figure 4), transformants containing pTrc-PST recombinants expressed an obvious 32 kDa subunit protein observed after Coomassie Blue R-250 staining that was immunoreactive with anti-(bovine PST) antiserum. Preliminary assays of the cytosolic extracts indicated no PST activity in the vector control transformants, whereas the pTrc-PST-containing cells were very reactive (results not shown). The expressed bovine PST was further characterized by its steady-state kinetic properties (Figure 5). The enzyme displayed simple saturation kinetics up to a substrate concentration of 50 $\mu$M 2-naphthol, with an approximate $K_m$ value of 2.7 $\mu$M, followed by substrate inhibition (Figure 5a). Further analyses with a narrower range of substrate concentrations (1–10 $\mu$M) revealed an apparent $K_m$ for 2-naphthol of 2.9 $\mu$M (Figure 5b). A replicate analysis with a separate extract indicated an apparent $K_m$ for 2-naphthol of 2.5 $\mu$M (not shown). Both the substrate inhibition pattern and the apparent $K_m$ value are consistent with the properties of a PST expressed by cultured bovine bronchial epithelial cells [13].

## PST transcript levels are variable along the respiratory tract

PST activity and immunoreactivity are highly variable along the bovine respiratory tract, being least in the tracheobronchial epithelia and greatest in distal or parenchymal tissue [26]. To determine if PST transcript levels also followed this pattern, RNA was extracted from tracheal, second generation bronchial and parenchymal lung tissues. Northern blot hybridization analysis using the bovine cDNA as a probe indicated low levels of PST transcripts in the large airways relative to the distal tissue (Figure 6). Scanning densitometry indicated relative 6.6-fold (lung 1) and 10-fold (lung 2) greater PST mRNA levels in the distal lung parenchyma compared with the tracheobronchial samples.

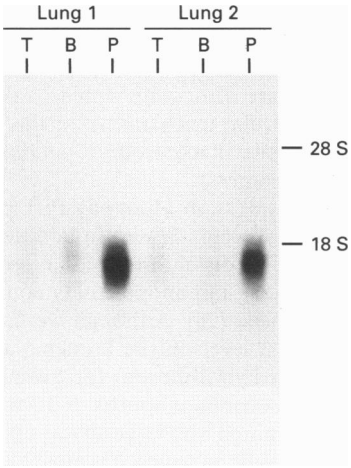


**Figure 6 Northern blot hybridization analysis of bovine lung RNA isolated at various tissue locations**

Each lane contained 20 $\mu$g of total RNA isolated from tracheal (T), second generation bronchial (B) and distal parenchymal (P) tissues. Positions of the ribosomal RNAs are indicated based on the ethidium stained-gel prior to blotting. Final washing was in 0.1 × SSPE with 0.1% SDS at 42 °C prior to autoradiography.

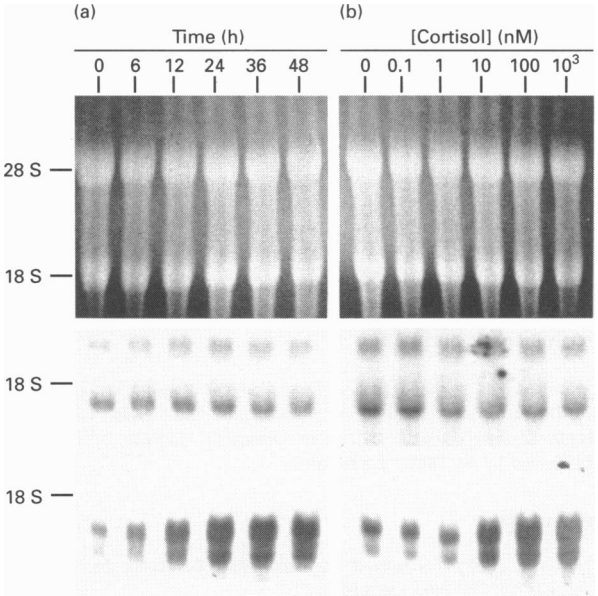


**Figure 7 Cortisol increases PST transcript levels expressed by cultured bovine bronchial epithelial cells**

Primary cells (see the Materials and methods section) were exposed to 100 nM cortisol for the indicated times (**a**) or to the indicated concentrations of cortisol for 48 h (**b**) prior to RNA extractions. Each lane contained 10 $\mu$g of total RNA. The upper panels of each set are ethidium bromide-stained; the gradients in fluorescence intensity towards the centre are an artifact of illumination. The centre panels were taken from replicate blots probed with $\beta$-tubulin cDNA as a loading control (see the materials and methods section). The bottom panels show the PST transcripts. Final washings were in 0.1 × SSPE with 0.1% SDS at 42 °C prior to autoradiography.

### PST transcript levels are regulated by cortisol in cultured bronchial epithelial cells

PST activity and protein levels are stimulated by cortisol within a bovine bronchial epithelial cell culture system [13]. To determine if cortisol also increases PST transcript levels within this model, primary bronchial epithelial cells were exposed to cortisol for various times and at various concentrations. Extracted RNA was then subjected to Northern blot hybridization analysis using the bovine PST cDNA as a probe (Figure 7); mouse $\beta$-tubulin cDNA was also used as a control to test for total RNA load equivalence between lanes. This control probe detected two bovine bronchial tubulin transcripts as previously reported [44], both of which indicated equal loading. In response to 100 nM cortisol, PST transcript levels were visibly increased after 6 h, with a maximal 5-fold increase (normalized to tubulin) at 24 h. For the various doses of cortisol, stimulation was obvious at 10 nM or greater, with a normalized 5–6-fold increase with 1 $\mu$M steroid. It is interesting that two transcript sizes were observed in both resting and stimulated cells, and the ratio of these species did not change in response to glucocorticoid. It is not clear at this time if these two sizes of transcripts are products of different genes or are due to variable transcriptional initiation or termination sites. Two transcripts have been reported for the rat minoxidil sulphotransferase from rat liver [28], and it has been proposed that this diversity is due to two 3′ polyadenylation signal sequences that have no impact on the translated protein product.

## DISCUSSION

In this investigation we have used a heterologous rat PST cDNA to isolate two bovine tracheal cDNA clones. Since these were partial non-overlapping sequences, oligonucleotides corresponding to the 5′ and 3′ ends of the anticipated complete coding region for the bovine PST were used to amplify the contiguous cDNA from the bovine tracheal epithelial cDNA library. The open reading frame predicts an amino acid sequence that matches perfectly with six tryptic peptide sequences isolated from the purified protein, and antibodies directed against the N-terminus predicted by the cDNA sequence bound to the PST purified from bovine lung. The identity of the sequence was confirmed by expression of active PST in *E. coli*. Whether this is the only bovine PST isoform is currently unknown. The first sulphotransferase cDNA sequence reported was for the bovine oestrogen sulphotransferase [41], and our bovine PST sequence shares 55% nucleotide and 50% amino acid sequence identities with this enzyme. This is greater divergence than in comparisons with the two human PST sequences (see below).

### Structural comparison of PSTs

An astounding feature to arise from these investigations is the degree of sequence conservation between and within species. Consequently, it is possible to begin to focus on specific structural domains that necessarily confer unique phenolic substrate specificities. For the two human PSTs, which are 93% identical [42], there exist only two domains of significant structural variance (Figure 8, arrows). For the human thermolabile (TL)-PST these regions are very hydrophilic in comparison with the thermostable (TS)-PSTs, whether human or bovine. The preferred substrates for the TL-PST are the positively charged catecholamines [45]. Therefore the substrate binding domain must necessarily accommodate such hydrophilic molecular properties. In contrast, both human and bovine TS-PSTs are relatively inactive with catecholamines, but prefer simple and hydrophobic phenolic compounds [16,39,46]. We propose that the two hydrophobic domains present in the TS-PSTs that are distinct from the TL-PST form the binding site for the acceptor substrate. It will be important to test this hypothesis, possibly using a recombinant molecular biological approach.

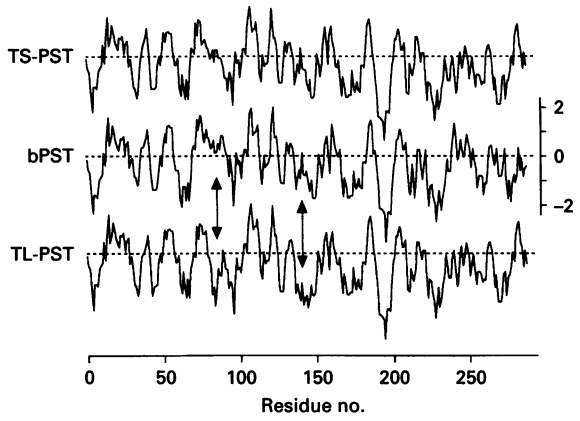


**Figure 8 Comparison of the predicted bovine and two human PST proteins by hydropathy analysis**

The dashed line through each profile is zero using the Kyte–Doolittle algorithm with a 7-amino-acid-residue window. Positive values indicate hydrophobic domains, whereas negative deflections reveal hydrophilic regions. The two arrows highlight the regions of greatest difference between the human TS-PST and TL-PST. The centre plot of the bovine PST (bPST) appears most similar to the human TS-PST in comparison.

It seems possible that the above structural variations account for the overall thermostability of the two classes of PSTs. Given the increased hydration to be expected with additional hydrophilic domains on the TL-PST, a decreased temperature required for structural unfolding compared with the hydrophobic TS-PSTs would be predicted.

We also propose that these significant differences account for the altered electrophoretic mobilities in the presence of SDS, despite the nearly equivalent predicted molecular masses of 34 kDa [40,42,47]. The TS-PSTs, both human and bovine, migrate at 32 kDa relative to standard markers [48]; the TL-PST has an apparently greater relative mass of 34–35 kDa [42,49], which is in closest agreement with that predicted by the cDNA sequence. The additional hydrophobic domains of the TS-PST proteins would be anticipated to bind an increased number of dodecyl sulphate molecules relative to the TL-PST protein. This would consequently increase the charge-to-mass ratio of the TS-PST/detergent complexes relative to the denatured TL-PST, thereby increasing their relative electrophoretic mobilities.

### Implications for the regulation of PSTs

We have recently determined that one natural and potent inhibitor of bovine lung PST is pyridoxal phosphate, which binds covalently to the enzyme, probably at the phenol binding site [12]. While experiments have not been conducted to test human PSTs for similar inhibition, the high degree of identity between the bovine and human enzymes (Figure 8, and results not shown) indicates the likelihood of such sensitivity to pyridoxal phosphate. It will be important to examine this question in greater detail, since such metabolic regulation could modify steady-state concentrations of active tri-iodothyronine [50], oestradiol [51] and monoamine neurotransmitters [52] in addition to modulating the rate of metabolism of numerous drugs or other xenobiotics.

### Controlled expression of bovine PST

We have previously shown that cortisol stimulates the expression of PST activity and protein within a bronchial epithelial cell culture model [13]. Here we demonstrate that nanomolar cortisol concentrations also stimulate steady-state levels of PST mRNA. The time courses for the stimulation of protein/enzyme levels versus mRNA levels are also quite similar, taking place over 12–48 h [13]. The molecular mechanisms for this stimulation are uncertain at this time and will require more detailed investigations at the gene level (in progress).

Regulation of the expression of airway PST protein [13] and mRNA levels probably occurs *in vivo* in addition to the above cell culture system. The distal bronchiolar epithelium shows much greater histological immunopositivity compared with the tracheobronchial epithelia [26]. Although we have not demonstrated elevated mRNA levels in the bronchiolar epithelium of bovine lung by *in situ* hybridization, for example, total distal lung parenchymal RNA contains approx. 6–10-fold greater levels of PST transcripts compared with large airway epithelium (Figure 6). Since PSTs are cytosolic enzymes, it is most likely that mRNA levels will closely parallel expressed enzyme levels within a particular cell type. Determination of the factor(s) that controls PST mRNA and protein expression within the various epithelial cell types along the respiratory tract will be aided by ongoing investigations into the PST gene(s). Regardless, the dynamic expression is clearly implicated by these results.

The bovine respiratory system is metabolically less complex than human lung with regard to sulphotransferase-mediated conjugation pathways. The former contains a thermostable enzyme that reacts with simple phenol substrates [39,46]. Human lung probably contains both the TS-PST and the TL-PST enzyme that sulphonates phenolic monoamines, such as noradrenaline [21,46]. Therefore experiments with the bovine system have been useful as a less complex experimental system [13]. However, it will be important to closely examine human airways with particular attention to the two types of PST isoforms. It will also be important to assess the roles of these enzymes, for example, in airway drug metabolism or in metabolic defence against cytotoxic xenobiotics. The present investigation provides valuable information about how the expression of these enzymes can be controlled, and will therefore aid in such pharmacological and toxicological experiments.

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## REFERENCES

1 Roy, A. B. (1981) in Sulfation of Drugs and Related Compounds (Mulder, G. J., ed.), pp. 83–130, CRC Press, Boca Raton, FL
2 Hashimoto, Y., Orellana, A., Gil, G. and Hirschberg, C. B. (1992) J. Biol. Chem. **267**, 15744–15750
3 Mulder, G. J. (1981) in Sulfation of Drugs and Related Compounds (Mulder, G. J., ed.), pp. 1–3. CRC Press, Boca Raton, FL
4 Buhl, A. E., Waldon, D. J., Baker, C. A. and Johnson, G. A. (1990) J. Invest. Dermatol. **95**, 553–557
5 Luks, H. J., Spratt, T. E., Vavrek, M. T., Roland, S. F. and Weisburger, J. H. (1989) Cancer Res. **49**, 4407–4411
6 Galinsky, R. E., Manning, B. W., Kimura, R. E. and Franklin, M. R. (1992) Exp. Gerontol. **27**, 221–232
7 Dalhoff, K. and Poulsen, H. E. (1993) Biochem. Pharmacol. **46**, 383–388
8 DeBaun, J. R., Miller, E. C. and Miller, J. A. (1970) Cancer Res. **30**, 577–595
9 Surf, Y.-J., Kwon, H. and Tannenbaum, S. R. (1993) Cancer Res. **53**, 1017–1022
10 Ringer, D. P., Norton, T. R. and Howell, B. A. (1990) Cancer Res. **50**, 5301–5307
11 Roochvarg, L. B., Thurman, R. G. and Kauffman, F. C. (1992) Dev. Pharmacol. Ther. **18**, 81–88
12 Bartzatt, R. and Beckmann, J. D. (1994) Biochem. Pharmacol. **47**, 2087–2095
13 Beckmann, J. D., Illig, M. and Bartzatt, R. (1994) J. Cell. Physiol. **160**, 603–610
14 Runge-Morris, M. and Wilusz, J. (1991) Biochem. Biophys. Res. Commun. **175**, 1051–1056

15 Ozawa, S., Nagata, K., Gong, D.-W., Yamazoe, Y. and Kato, R. (1993) Jpn. J. Pharmacol. **61**, 153–156
16 Campbell, N. R. C., Van Loon, J. A., Sundaram, R. S., Ames, M. M., Hansch, C. and Weinshilboum, R. (1987) Mol. Pharmacol. **32**, 813–819
17 Sekura, R. D., Duffel, M. W. and Jakoby, W. B. (1981) Methods Enzymol. **77**, 197–206
18 Anderson, R. J. and Jackson, B. L. (1984) Clin. Chim. Acta **138**, 185–196
19 Bostrom, H. and Wengle, B. (1967) Acta Endocrinol. **56**, 691–704
20 Foldes, A. and Meek, J. L. (1974) J. Neurochem. **23**, 303–307
21 Baranczyk-Kuzma, A. (1986) Biochem. Med. Metab. Biol. **35**, 18–30
22 Wells, M. S. and Nerland, D. E. (1991) Toxicol. Lett. **56**, 159–166
23 Cassidy, M. K. and Houston, J. B. (1980) Biochem. Pharmacol. **29**, 471–474
24 Gibby, E. M. and Cohen, G. M. (1984) Biochem. Pharmacol. **33**, 739–743
25 Autrup, H., Wefald, F., Jeffrey, A. M., Tate, H., Schwartz, R. D., Trump, B. F. and Harris, C. C. (1980) Int. J. Cancer **25**, 293–300
26 Beckmann, J. D., Spurzem, J. R. and Rennard, S. I. (1993) Cell Tissue Res. **274**, 475–485
27 Beckmann, J. D., Takizawa, H., Romberger, D., Illig, M., Claassen, L., Rickard, K. and Rennard, S. I. (1992) In Vitro Cell. Dev. Biol. **28A**, 39–46
28 Hirshey, S. J., Dooley, T. P., Reardon, I. M., Heinrikson, R. L. and Falany, C. N. (1992) Mol. Pharmacol. **42**, 257–264
29 Ozawa, S., Nagata, K., Gong, D., Yamazoe, Y. and Kato, R. (1990) Nucleic Acids Res. **18**, 4001
30 Sekura, R., Marcus, C. J., Lyon, E. S. and Jakoby, W. B. (1979) Anal. Biochem. **95**, 82–86
31 Beckmann, J. D. (1991) Anal. Biochem. **197**, 408–411
32 Laemmli, U. K. (1970) Nature (London) **227**, 680–685
33 Matsudaira, P. (1987) J. Biol. Chem. **262**, 10035–10038
34 Choli, T., Kapp, U. and Wittmann-Liebold, B. (1989) J. Chromatogr. **476**, 59–72
35 Chomczynski, P. and Sacchi, N. (1987) Anal. Biochem. **162**, 156–159
36 Maniatis, T., Fritsch, E. F. and Sambrook, J. (1982) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor
37 Feinberg, A. P. and Vogelstein, B. (1984) Anal. Biochem. **137**, 266–267
38 Towbin, H., Staehelin, T. and Gordon, J. (1979) Proc. Natl. Acad. Sci. U.S.A. **76**, 4350–4354
39 Baranczyk-Kuzma, A. and Szymczyk, T. (1987) Biochem. Pharmacol. **36**, 3141–3146
40 Wilborn, T. W., Comer, K. A., Dooley, T. P., Reardon, I. M., Heinrikson, R. L. and Falany, C. N. (1993) Mol. Pharmacol. **43**, 70–77
41 Nash, A. R., Glenn, W. K., Moore, S. S., Kerr, J., Thompson, A. R. and Thompson, E. O. P. (1988) Aust. J. Biol. Sci. **41**, 507–516
42 Wood, T. C., Aksoy, I. A., Aksoy, S. and Weinshilboum, R. M. (1994) Biochem. Biophys. Res. Commun. **198**, 1119–1127
43 Otterness, D. M., Wieben, E. D., Wood, T. C., Watson, R. W. G., Madden, B. J., McCormick, D. J. and Weinshilboum, R. M. (1992) Mol. Pharmacol. **41**, 865–872
44 Romberger, D. J., Beckmann, J. D., Claassen, L., Ertl, R. F. and Rennard, S. I. (1992) Am. J. Respir. Cell Mol. Biol. **7**, 149–155
45 Sundaram, R. S., Szumlanski, C., Otterness, D., Van Loon, J. A. and Weinshilboum, R. M. (1989) Drug Metab. Disp. **17**, 255–264
46 Baranczyk-Kuzma, A. and Szymczyk, T. (1986) Biochem. Pharmacol. **35**, 995–999
47 Zhu, X., Veronese, M. E., Bernard, C. C. A., Sansom, L. N. and McManus, M. E. (1993) Biochem. Biophys. Res. Commun. **195**, 120–127
48 Falany, C. N., Vazquez, M. E., Heroux, J. A. and Roth, J. A. (1990) Arch. Biochem. Biophys. **278**, 312–318
49 Heroux, J. A., Falany, C. N. and Roth, J. A. (1989). Mol. Pharmacol. **36**, 29–33
50 Otten, M. H., Mol, J. A. and Visser, T. J. (1983) Science **221**, 81–83
51 Aksoy, I. A., Wood, T. C. and Weinshilboum, R. (1994) Biochem. Biophys. Res. Commun. **200**, 1621–1629
52 Whittemore, R. M., Pearce, L. B. and Roth, J. A. (1985) Biochemistry **24**, 2477–2482

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