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CHOLESTEROL SULFATION IN HUMAN LIVER

Catalysis by Dehydroepiandrosterone Sulfotransferase

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ABSTRACT:

Cholesterol can undergo sulfate conjugation to form cholesterol 3-sulfate. Our experiments were performed to determine whether human liver cytosol could catalyze the sulfation of cholesterol, and, if so, whether any of the three well-characterized human hepatic cytosolic sulfotransferases, dehydroepiandrosterone sulfotransferase (DHEA ST), thermostable (TS) phenol sulfotransferase (PST), or thermolabile (TL) PST might participate in the reaction. On the basis of substrate kinetics, two "forms" of cholesterol sulfotransferase (CST) activity were present in human liver cytosol, one with high and one with low affinity for cholesterol. Apparent K_M values of the high- and low-affinity activities were 0.14 and 15 μM for cholesterol and 0.30 and 0.19 μM for 3′-phosphoadenosine-5′-phosphosulfate, respectively. Both kinetic forms of CST activity had thermal inactivation profiles similar to those of DHEA ST and TS PST, but both were more thermostable than was TL PST. Enzyme inhibition studies performed with 2,6-dichloro-4-nitrophenol (DCNP) showed that inhibition profiles for both high- and low-affinity CST activities were similar to those of DHEA ST and TL PST, but both were more resistant to DCNP inhibition than was TS PST. Experiments performed with 20 individual human liver samples confirmed these observations and demonstrated highly significant correlations between both high- and low-affinity CST activities and DHEA ST activity (r_s = 0.740, p = 0.0001 and r_s = 0.767, p < 0.0001, respectively). However, the level of activity of neither kinetic form of CST activity was significantly correlated with either TS or TL PST activities. Finally, COS-1 cells transfected with human liver DHEA ST cDNA expressed both high- and low-affinity CST activities with biphasic substrate kinetics for cholesterol similar to those found in human hepatic cytosol. All of these results were compatible with the conclusion that the sulfation of cholesterol can occur in human liver cytosol and that DHEA ST is the major enzyme that catalyzes that reaction.

Sulfate conjugation is an important pathway in the biotransformation of many drugs, xenobiotics, hormones, and endogenous compounds (1, 2). Cholesterol can undergo sulfate conjugation (3, 4), and the sulfation of cholesterol may play a role in the differentiation of epithelial cells (5–7), the integrity of lipid membranes (8), the capacitation of sperm (9), and the pathophysiology of X-linked ichthyosis (3). Studies of the enzyme or enzymes that catalyze cholesterol sulfation have been performed previously with cultured epidermal keratinocytes and with bronchial epithelial cells (5–7, 10). However, the liver is a major site for both drug and cholesterol metabolism and a great deal is known about ST[1] enzymes in human liver. Therefore, it would be important to determine whether human hepatic tissue can catalyze the sulfation of cholesterol, and, if so, to determine which enzyme or enzymes might be involved.

Human liver contains three well-characterized cytoplasmic ST enzymes, DHEA ST (11–13) and two forms of PST (14, 15). These three enzymes differ in their physical properties, substrate specificities, inhibitor sensitivities, and regulation among individuals. One form of PST is thermolabile (TL), catalyzes the sulfate conjugation of micromolar concentrations of dopamine and other catechol or phenolic monoamines, and is resistant to inhibition by DCNP (14–21). The other form is thermostable (TS), catalyzes the sulfate conjugation of micromolar concentrations of "simple" phenols such as *p*-nitrophenol, and is sensitive to inhibition by DCNP. DHEA ST catalyzes the sulfate conjugation of bile acids and steroid hormones such as estrogens, is relatively thermostable, and is resistant to DCNP inhibition (11–13). However, there is significant overlap in the substrate specificities of these three enzymes because *p*-nitrophenol at millimolar concentrations can serve as a substrate for TL PST (19, 21), dopamine at millimolar concentrations can serve as a substrate for TS PST (14), and TS PST is capable of catalyzing the sulfation of steroid hormones such as 17β-estradiol and estrone (22). The present experiments were performed to determine whether human liver cytosol could catalyze the sulfate conjugation of cholesterol, and, if so, to study the characteristics of the enzyme or enzymes involved in that reaction.

Materials and Methods

Tissue Acquisition and Preparation. Hepatic tissue was obtained from patients who underwent clinically indicated hepatectomies performed for the removal of primary or metastatic hepatic tumors. All tissue was obtained under guidelines approved by the Mayo Clinic Institutional Review Board. Tissue that was normal by gross pathologic examination was either stored at −80°C or was homogenized in 9 volumes of 5 mM potassium phosphate buffer (pH 7.5), followed by the preparation of a 100,000*g* supernatant as described previously (14). TS and TL PST activities have been demonstrated previously to be stable in human liver samples stored at −80°C (23). The 100,000*g* supernatant preparations were then centrifuged at 300,000*g* for 6.5 hr at 4°C to prepare an HSS. HSS was used in our experiments because Rearick *et al.* (5) reported that, in other tissues, HSS gave higher CST activity than did 100,000*g* supernatant preparations. HSS preparations from 10 individual liver samples were pooled, and this pooled material was used to determine

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[1] Abbreviations used are: ST, sulfotransferase; DHEA ST, dehydroepiandrosterone sulfotransferase; PST, phenol sulfotransferase; TL, thermolabile; DCNP, 2,6-dichloro-4-nitrophenol; TS, thermostable; HSS, high-speed supernatant; CST, cholesterol sulfotransferase; ^{35}S-PAPS, ^{35}S-3′-phosphoadenosine-5′-phosphosulfate.

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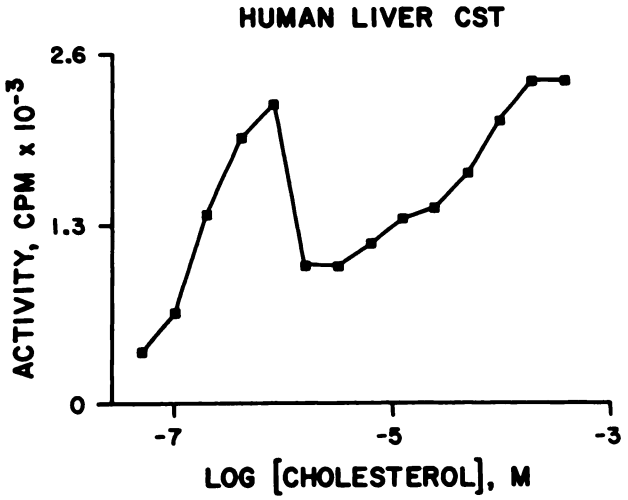

FIG. 1. *Effect of cholesterol concentration on human liver CST activity.*

Data are plotted in a semilogarithmic fashion because of the wide range of cholesterol concentrations studied. Each point is the mean of three determinations.

optimal conditions for the assay of human liver CST activity as well as for thermal inactivation and DCNP inhibition experiments. Correlation studies were performed with HSS preparations from 20 individual liver samples.

CST Assays. Initial experiments showed that both high- and low-affinity CST activities were present in human liver cytosol. Therefore, assay conditions for the measurement of each of these activities were determined separately. The assay procedure used was a modification of the method of Rearick *et al.* (5), with cholesterol as the sulfate acceptor and ^{35}S-PAPS as the sulfate donor. With the exception of cholesterol concentration and dilution of the tissue preparation, the assays used to measure low- and high-affinity CST activities were identical. Specifically, HSS was diluted 1:133 (v/v) for the assay of low-affinity and 1:50 (v/v) for the assay of high-affinity CST activity, respectively. Cholesterol was dissolved in 5 μl of isopropanol, and the final concentrations of cholesterol present during the assay were 200 or 0.5 μM for the measurement of low- or high-affinity CST activities, respectively. In both cases, the reaction pH in the presence of 8.2 mM potassium phosphate buffer was 7.4; the ^{35}S-PAPS concentration was 0.4 μM; and the Mg^{2+} concentration was 0.75 mM. Blanks were samples that contained isopropanol but no cholesterol. After a 60-min incubation at 37°C, enzyme reactions were terminated by the addition of 4 ml of a mixture of chloroform and methanol (2:1, v/v), followed by the addition of 1 ml of 0.1 M KCl. Reaction tubes were then vortexed twice for 15 sec and were centrifuged at 600*g* for 5 min to separate aqueous and organic phases. The aqueous phase was discarded, and the organic phase was extracted a second time by the addition of 1 ml of methanol and 1 ml of 0.1 M KCl. A 2.5 ml aliquot of the organic phase was then transferred to a glass liquid scintillation counting vial, and was evaporated to dryness in a ventilated fume hood. Ten ml of Bio-Safe II liquid scintillation counting fluid was added to the vial, and radioactivity was measured. All results were corrected for quench and counting efficiency. One unit of enzyme activity represented the formation of 1 nmole of sulfated product per hr of incubation at 37°C. All assays were performed in triplicate, and values reported are averages of those three determinations.

DHEA ST, TS PST, and TL PST Assays. DHEA ST, TS PST, and TL PST activities were assayed with the method of Foldes and Meek (24), as modified by Hernández *et al.* (22) to measure DHEA ST activity

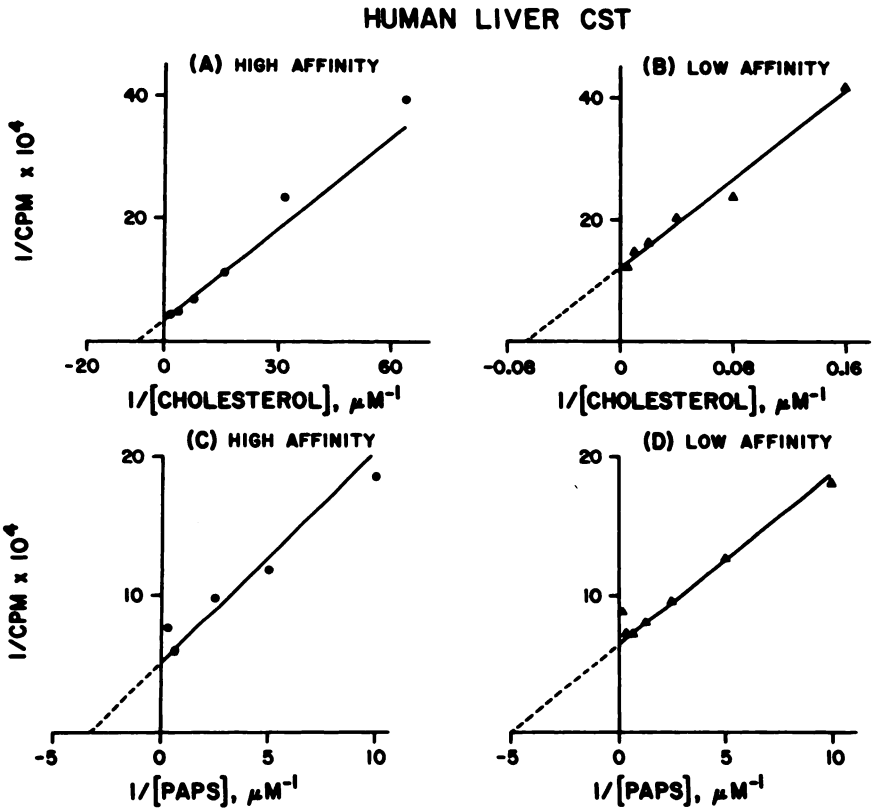

FIG. 2. *Effect of cholesterol and PAPS concentrations on high- and low-affinity human liver CST activities.*

Double inverse plots are shown for (*A*) cholesterol concentration vs. reaction velocity for high-affinity CST activity, (*B*) cholesterol concentration vs. reaction velocity for low-affinity CST activity, (*C*) PAPS concentration vs. reaction velocity for high-affinity CST activity, and (*D*) PAPS concentration vs. reaction velocity for low-affinity CST activity. Each point is the mean of three determinations.

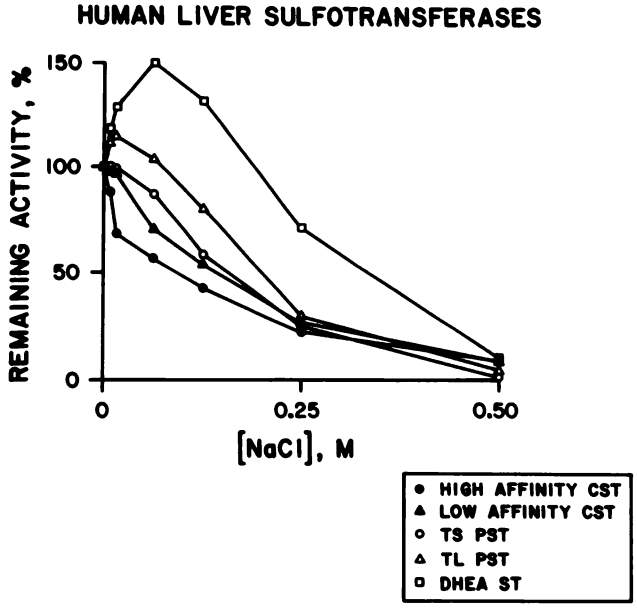

FIG. 3. *Effect of NaCl concentration on human liver ST activities.*

Each point is the mean of three determinations.

and by Campbell *et al.* (14) to measure TS and TL PST activities in human liver cytosol preparations. The sulfate acceptor substrates were 5 μM DHEA, 4 μM *p*-nitrophenol, and 60 μM dopamine in the DHEA ST, TS PST, and TL PST assays, respectively. The concentration of ^{35}S-PAPS used in all three assays was 0.4 μM.

Protein Assay. Protein concentrations were measured by the dye binding method of Bradford (25), with bovine serum albumin as a standard.

HPLC Identification of Reaction Products. The product of the CST reaction was identified by HPLC performed at room temperature with a Waters 3.9 mm × 30 cm μBondaPak C_{18} reversed-phase column. The mobile phase consisted of 2 mM *n*-octylamine in acetonitrile and water (55:45) at a flow rate of 1 ml/min. UV absorbance was monitored at 200 nm. Prior to HPLC analysis, enzyme reactions were performed in the usual fashion, and sample extracts that had been evaporated to dryness were dissolved in the mobile phase. Fifty μl of this solution was injected onto the reversed-phase HPLC column. Fractions of HPLC eluant were collected every 30 sec, 10 ml of Bio-Safe II liquid scintillation counting fluid was added, and radioactivity was measured.

Thermal Stability Experiments. Thermal stabilities of ST activities were measured as described previously (18, 19). Specifically, aliquots of pooled HSS diluted 1:25 (v/v) in 5 mM potassium phosphate buffer (pH 6.5) were preincubated at various temperatures for 15 min and were placed on ice. An aliquot of the same preparation was kept at 4°C as a control. No substrate or other constituent of the reaction mixture was present during the thermal treatment. Each aliquot was then diluted appropriately for assay of the ST activity being measured. Values for blank samples were determined at each temperature studied.

Expression of DHEA ST cDNA in COS-1 Cells. Human liver DHEA ST cDNA was expressed in COS-1 cells by use of the expression construct 17G described by Otterness *et al.* (12). 17G was constructed by cloning a DHEA ST cDNA (clone G) into the Eco*RI* site of the expression vector P91023(B) (12, 26, 27) and was transiently expressed in COS-1 cells as described previously (12). HSS was prepared from these homogenates, and high- and low-affinity CST and DHEA ST activities were measured in 20 μl aliquots under the assay conditions previously described.

Data Analysis. The results of correlation studies were analyzed by Spearman rank correlation coefficients by the use of NWA Statpak (Northwest Analytical, Inc., Porland, OR). Apparent Michaelis (K_M) constants were calculated by the method of Wilkinson (28), with a computer program written by Cleland (29). Data obtained during NaCl inhibition, thermal inactivation, and DCNP inhibition studies were analyzed with the GraphPAD InPlot curve-fitting program (GraphPAD InPlot Software, San Diego, CA).

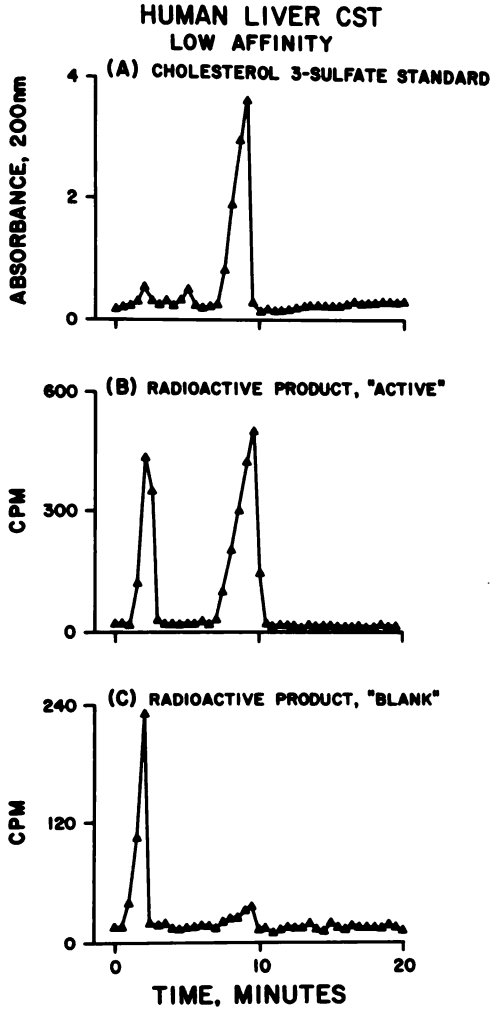

FIG. 4. *HPLC identification of the reaction product of the human liver low-affinity CST reaction.*

(*A*) Elution of a "standard" of cholesterol 3-sulfate. (*B*) Elution of radioactive reaction products from an "active" sample that included 200 μM cholesterol. (*C*) Elution of radioactivity present in a "blank" sample that included no exogenous cholesterol. Note the difference in scales for the y-axes of (*B*) and (*C*). CPM, counts per minute.

Materials. ^{35}S-PAPS (1.5–2.5 Ci/mmol) was purchased from New England Nuclear Corporation (Boston, MA). KCl, *p*-nitrophenol, *n*-octylamine, Triton X-100, dopamine-HCl, DHEA, and cholesterol (grade I, from porcine liver) were obtained from Sigma Chemical Company (St. Louis, MO). Cholesterol 3-sulfate was purchased from Steraloids, Inc. (Wilton, NH). HPLC grade isopropanol was obtained from Mallinckrodt (Paris, KY), and HPLC grade acetonitrile was purchased from Baxter Healthcare Corporation (Muskegon, MI). Bio-Safe II was obtained from Research Products International Corp. (Mount Prospect, IL). COS-1 cells were purchased from American Type Culture Collection (Rockville, MD).

Results

Human Liver CST Activity. *Effect of Cholesterol Concentration.* The effect of cholesterol concentration on CST activity was determined. Two separate peaks of enzymatic activity were present, with maximal activities at cholesterol concentrations of approximately 0.5 and 200 μM, respectively (fig. 1). The high-

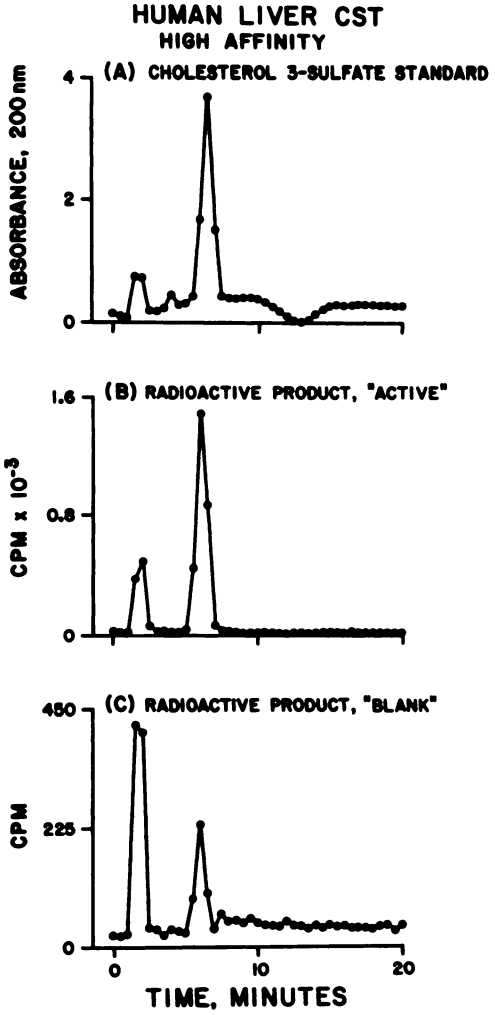

FIG. 5. *HPLC identification of the reaction product of the human liver high-affinity CST reaction.*

(*A*) Elution of a "standard" of cholesterol 3-sulfate. (*B*) Elution of radioactive reaction products from an "active" sample that included 0.5 μM cholesterol. (*C*) Elution of radioactivity present in a "blank" sample that included no exogenous cholesterol. Note the differences in scales for the y-axes of (*B*) and (*C*). CPM, counts per minute.

affinity activity displayed apparent substrate inhibition—a phenomenon that has been observed previously with DHEA ST, TS PST, and TL PST activities (11, 17, 18, 22). Apparent K_M values of the high- and low-affinity CST activities for cholesterol calculated from double reciprocal plots (fig. 2, *A* and *B*) were 0.14 and 15 μM, respectively. The data used to perform these calculations were only those obtained with cholesterol concentrations at which there was no evidence of substrate inhibition (fig. 2, *A* and *B*). In all subsequent experiments, the concentrations of cholesterol used to assay high- and low-affinity human liver CST activities were 0.5 and 200 μM, respectively.

Effect of PAPS Concentration. The effect of increasing concentrations of PAPS on activities of the two kinetic forms of CST present in human liver cytosol were then determined in the presence of 0.5 and 200 μM cholesterol. Double reciprocal plots were constructed with these data (fig. 2, *C* and *D*), and apparent K_M values of high- and low-affinity CST activities for PAPS were calculated to be 0.30 and 0.19 μM, respectively. In all subsequent assays, a PAPS concentration of 0.4 μM was used to measure both CST activities. The use of higher concentrations of PAPS resulted in an increase in counts per minute in blank samples, and, therefore, a decrease in the sensitivity of the assay. Similar observations have been made previously during studies of DHEA ST, TS PST, and TL PST activities in human liver cytosol preparations (14, 22). Because our assays, like those performed in the course of most previous studies of ST activities in human tissue (14, 17, 18, 22), were not conducted with saturating concentrations of PAPS, the results reported subsequently can only be compared directly with data obtained with identical concentrations of PAPS.

Effect of Tissue Quantity, Incubation Time, and pH. There was a linear relationship between HSS protein concentration and CST activity up to at least 15 μg of protein per sample for low-affinity and up to at least 25 μg of protein per sample for high-affinity CST activity. Subsequent assays were performed with approximately 5.6 and 15 μg of protein per sample for low- and high-affinity CST activities, respectively. There was also a linear relationship between enzyme activity and time of incubation for up to at least 90 min with both forms of CST activity. All subsequent assays were performed with an incubation time of 60 min. Maximal activities for both the high- and low-affinity CST activities were found at a reaction pH of 7.4 in the presence of 8.2 mM potassium phosphate buffer. This pH value was used in all subsequent assays.

Effect of Mg^{2+}. Human liver DHEA ST activity, unlike TS or TL PST activities, is increased by Mg^{2+} (11, 22). Therefore, human liver CST activities were measured in the presence of a series of concentrations of $MgCl_2$. The optimal Mg^{2+} concentration for both activities was approximately 0.75 mM. The maximal percentage increases in high- and low-affinity CST activities in the presence of 0.75 mM Mg^{2+} were 75% and 50%, respectively. All subsequent assays were performed in the presence of 0.75 mM $MgCl_2$.

Effect of NaCl and Triton X-100. CST activity measured in rabbit tracheal epithelial cells has been reported to be increased by NaCl and by Triton X-100 (5). Therefore, the effect of a series

TABLE 1

High- and low-affinity CST enzymatic activities in 100,000g supernatant and 300,000g supernatant (HSS) preparations of human liver cytosol

Sample	High-Affinity CST		Low-Affinity CST	
	100,000 × *g*	HSS	100,000 × *g*	HSS
Active	20,500 ± 700	110,900 ± 3,100	59,900 ± 2,300	321,700 ± 11,100
Blank	12,800 ± 800	31,000 ± 1,400	13,000 ± 600	44,200 ± 1,700
Net	7,700 ± 700	79,900 ± 3,100	46,900 ± 2,300	277,500 ± 11,200
Units/mg protein	0.005 ± 0.0004	0.05 ± 0.002	0.03 ± 0.001	0.17 ± 0.007

"Active" samples contained added cholesterol, whereas "blank" samples did not. Values shown are mean counts per minute per mg protein ± SEM for at least three determinations. The activities of each preparation are also shown as units/mg protein.

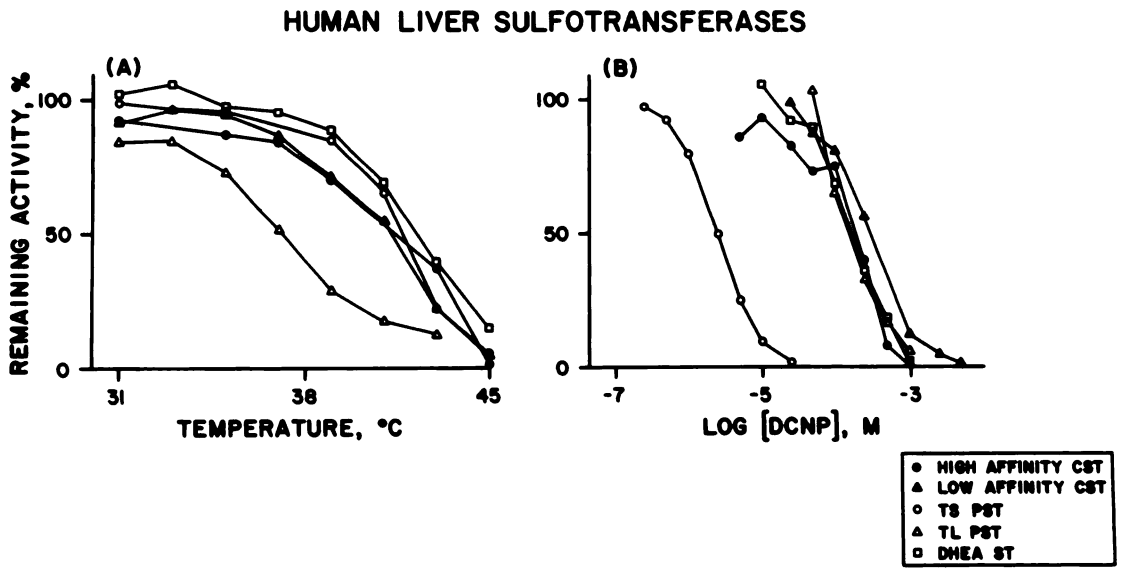

FIG. 6. *Human liver ST activity (A) thermal stabilities and (B) inhibition by DCNP.*

Each point is the mean of at least three determinations.

of concentrations of NaCl from 4 to 500 mM on human liver high- and low-affinity CST, DHEA ST, TS PST, and TL PST activities was determined (fig. 3). NaCl inhibited the activities of both kinetic forms of human liver CST at all concentrations tested. Fifty percent inhibition of high- and low-affinity CST activities occurred at NaCl concentrations of 117 ± 11.4 and 143 ± 15.4 mM (mean ± SEM), respectively. DHEA ST activity increased in the presence of lower NaCl concentrations, with a maximal increase of 50% at approximately 60 mM NaCl. Concentrations of NaCl of greater than 200 mM inhibited DHEA ST activity, with an IC_{50} of 277 ± 2.7 mM calculated on the basis of comparison with the basal, unactivated level of the enzyme activity (fig. 3). TS and TL PST activities were inhibited 50% by 161 ± 7.7 and 184 ± 1.8 mM NaCl, respectively. The effects of various concentrations of Triton X-100 from 0.0025% to 0.16% on high- and low-affinity CST activities were also tested. Both activities were inhibited approximately 75% at the lowest concentration of Triton X-100 studied, and exposure to higher concentrations resulted in even greater inhibition.

HPLC Identification of Reaction Products. The products of both the high- and low-affinity human liver CST reactions were identified as cholesterol 3-sulfate by C_{18} reversed-phase HPLC (figs. 4 and 5). HPLC analysis of the products of the two reactions was performed with two separate C_{18} reversed-phase columns, resulting in slightly different elution times from the two columns for a standard of cholesterol 3-sulfate. Blank samples with no exogenously added cholesterol contained a small peak that coeluted with cholesterol 3-sulfate (figs. 4*C* and 5*C*), a peak that probably represented the sulfation of endogenous cholesterol. In both cases, blank samples also contained a radioactive peak that eluted earlier than cholesterol 3-sulfate (figs. 4*C* and 5*C*). This material was assumed to result from the sulfation of an endogenous sulfate acceptor other than cholesterol. In all of the assays performed during our studies, radioactivity present in blank samples was subtracted from that present in "active" samples that contained cholesterol to control for the sulfation of endogenous compounds. However, for both the high- and low-affinity reactions, the major peak of radioactivity in active samples that contained exogenously added cholesterol coeluted with cholesterol 3-sulfate (figs. 4*B* and 5*B*).

Comparison of HSS with 100,000g Supernatant. Rearick *et al.* (5) reported much greater CST activity in 470,000*g* than in 100,000*g* supernatant preparations of rabbit tracheal epithelial cells. Therefore, our experiments were conducted with material obtained by centrifugation of human liver 100,000*g* supernatant preparations at 300,000*g* for 6.5 hr to produce HSS. After optimal CST assay conditions had been determined, it was possible to compare CST activity measured in 100,000*g* supernatant with that in HSS. The protein concentrations in the 100,000*g* supernatant and in the HSS preparations were 9.23 and 6.88 mg/ml, respectively. Table 1 shows that assays of both low- and high-affinity CST activities in HSS and in 100,000*g* supernatants from human liver confirmed the observations of Rearick *et al.* (5). High- and low-affinity human liver CST activities were approximately 10- and 6-fold greater in HSS than in 100,000*g* supernatant preparations, respectively. Therefore, all subsequent experiments, like all previous studies, were performed with HSS—the preparation in which optimal assay conditions had been determined. The next series of experiments was designed to compare the properties and regulation of CST in human liver with those of DHEA ST, TS PST, and TL PST.

Comparative Properties of Human Liver STs. *Thermal Stability Studies.* Thermal stability is a sensitive indicator of differences in protein structure (30, 31). The thermal stability of TL PST differs dramatically from those of DHEA ST and TS PST (14, 18, 21, 22). Therefore, the thermal stabilities of high- and low-affinity CST, DHEA ST, TS PST, and TL PST activities were compared in hepatic preparations that had been preincubated for 15 min at various temperatures as described in *Materials and Methods.* As anticipated, TL PST activity was much more thermolabile than was TS PST activity (fig. 6*A*). DHEA ST activity and both high- and low-affinity CST activities had thermal stabilities much closer to that of TS than of TL PST (fig. 6*A*). The estimated temperatures at which 50% inactivation occurred were 37.3 ± 0.18°C, 41.7 ± 0.06°C, 42.8 ± 0.35°C, 41.5 ± 0.71°C, and 41.7 ± 0.35°C (mean ± SEM) for TL PST, TS PST, DHEA ST, high-affinity CST, and low-affinity CST, respectively.

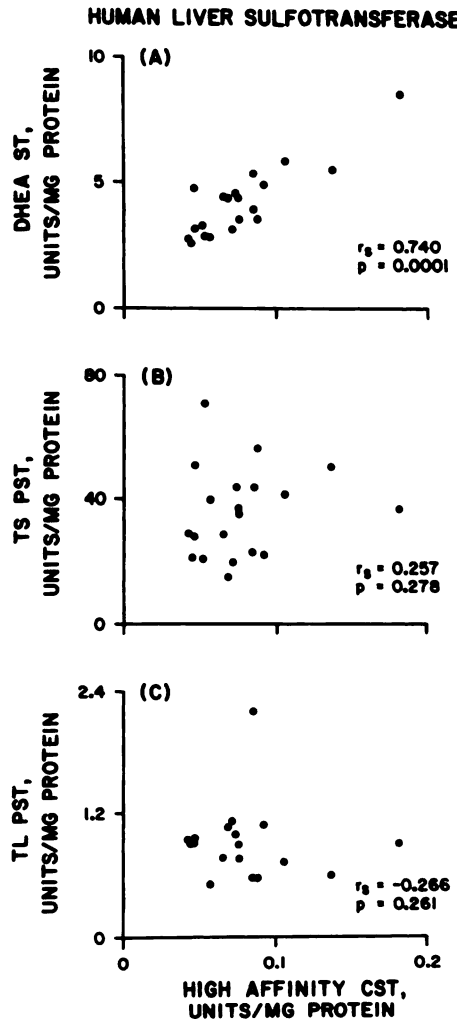

FIG. 7. *Correlation of ST activities in individual human liver samples.*

High-affinity CST activities are plotted vs. (*A*) DHEA ST activities, (*B*) TS PST activities, and (*C*) TL PST activities. Each point is the mean of three determinations.

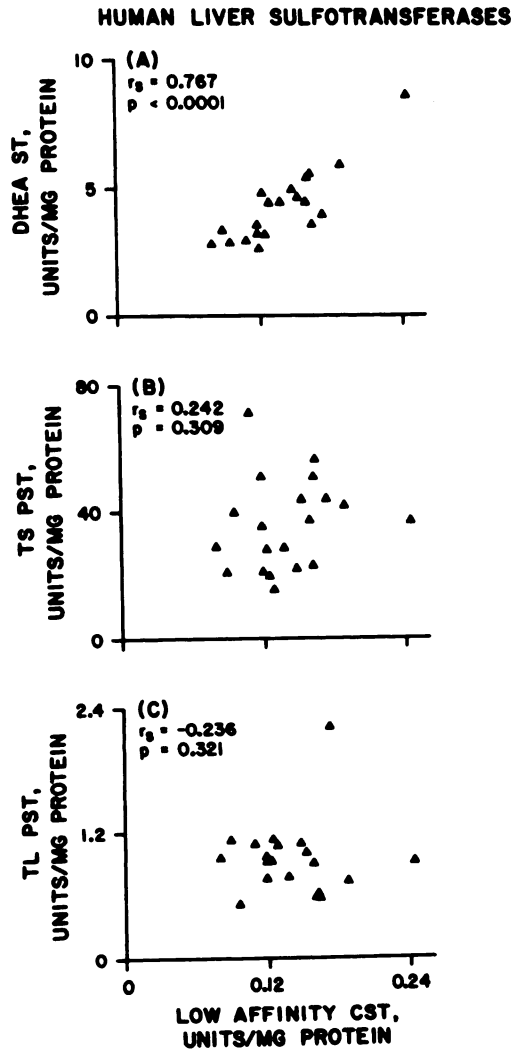

FIG. 8. *Correlation of ST activities in individual human liver samples.*

Low-affinity CST activities are plotted vs. (*A*) DHEA ST activities, (*B*) TS PST activities, and (*C*) TL PST activities. Each point is the mean of three determinations.

DCNP Inhibition Studies. TS PST is much more sensitive to inhibition by DCNP than are either TL PST or DHEA ST (13, 17, 20–22). Therefore, all five ST activities were measured in the presence of a series of concentrations of DCNP ranging from 0.00025 to 1.0 mM (fig. 6*B*). As anticipated, TS PST was much more sensitive to DCNP inhibition than were either TL PST or DHEA ST activities (fig. 6*B*). Both high- and low-affinity CST activities had DCNP inhibition profiles similar to those of DHEA ST and TL PST (fig. 6*B*). TS PST activity was found to be inhibited 50% at a DCNP concentration of approximately 2.5 ± 0.09 μM (mean ± SEM), whereas IC_{50} values for TL PST, DHEA ST, high-affinity CST and low-affinity CST were calculated to be 142 ± 20.6, 198 ± 25.7, 259 ± 19.7, and 376 ± 17.1 μM, respectively.

Individual Correlation Studies. There are large individual variations in TS PST, TL PST, and DHEA ST activities in human liver, and levels of these three ST enzyme activities are regulated independently (14, 18, 20, 22). We took advantage of this large individual variation to determine whether levels of high- and/or low-affinity CST activity might be regulated in parallel with those of DHEA ST, TS PST, or TL PST. All five ST activities were measured in 20 liver samples obtained from 11 female and 9 male patients. Average levels of DHEA ST, TS PST, TL PST, high-affinity CST, and low-affinity CST activities in these samples were 4.3 ± 0.32, 35.7 ± 3.2, 0.93 ± 0.08, 0.08 ± 0.007, and 0.14 ± 0.009 units/mg protein (mean ± SEM) when 5 μM DHEA, 4 μM *p*-nitrophenol, 60 μM dopamine, 0.5 μM cholesterol, and 200 μM cholesterol were used as substrates, respectively. The average values for human liver DHEA ST, TS PST, and TL PST activities obtained in this study were very similar to those reported during previous studies in which the same PAPS concentration and the same sulfate acceptor substrates were used to measure enzyme activities (14, 22, 32).

DHEA ST activities in these 20 human liver samples were significantly correlated with both high-affinity (r_s = 0.740, p = 0.0001) and low-affinity CST activities (r_s = 0.767, $p < 0.0001$) (figs. 7*A* and 8*A*). The two CST activities were also significantly correlated with each other (r_s = 0.854, $p < 0.0001$). However, neither of the human hepatic CST activities were correlated significantly with either TS or TL PST activities (figs. 7*B*, 7*C*, 8*B*, and 8*C*). As anticipated, TS PST activities were also not significantly correlated with either TL PST activities (r_s = −0.280, p = 0.236) or DHEA ST activities (r_s = −0.043, p =

TABLE 2

Expression of DHEA ST and high- and low-affinity CST enzymatic activities after transfection of COS-1 cells with DHEA ST cDNA

	DHEA ST	High-Affinity CST	Low-Affinity CST
P91023(B) (control)	0.32 ± 0.09	ND	ND
P91023(B) with DHEA ST cDNA (expression construct 17G)	8.17 ± 0.1	0.25 ± 0.03	0.19 ± 0.03

The P91023(B) control was expression vector that contained no insert. The expression construct 17G contained human liver DHEA ST cDNA as an insert. Activities were measured in HSS, and values shown are units/mg protein (mean ± SEM) for at least three determinations. ND indicates that no activity was detectable.

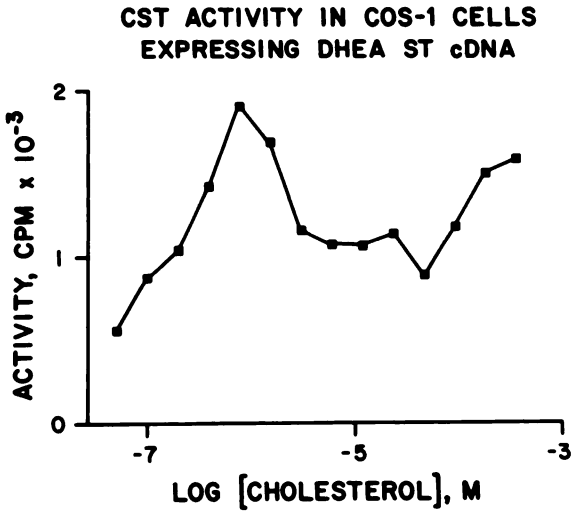

FIG. 9. *Effect of cholesterol concentration on CST activity in COS-1 cells in which human liver DHEA ST cDNA has been expressed.*

Data are plotted in a semilogarithmic fashion because of the wide range of cholesterol concentrations studied. Each point is the mean of three determinations.

0.857), nor were TL PST activities significantly correlated with DHEA ST activities ($r_s = -0.227$, $p = 0.341$).

DHEA ST cDNA Expression Studies. COS-1 cells were transfected with the expression construct 17G, a construct that consisted of human liver DHEA ST cDNA subcloned into the eukaryotic expression vector P91023(B). Control COS-1 cells were transfected with P91023(B) that contained no insert. DHEA ST and high- and low-affinity CST activities were then measured in HSS obtained from these cells (table 2). The assay conditions used were those determined to be optimal for the measurement of these enzyme activities in human liver preparations. DHEA ST enzymatic activity and both high- and low-affinity CST activities were present in COS-1 cells transfected with 17G, but no appreciable DHEA ST or CST activities could be detected in COS-1 cells transfected with P91023(B) that did not contain insert (table 2). After demonstrating that both CST activities were present in COS-1 cells that expressed human liver DHEA ST cDNA, CST activity was measured in HSS from these cells in the presence of a series of concentrations of cholesterol to determine whether DHEA ST might be responsible for the biphasic substrate curve for cholesterol in human liver HSS that is shown in fig. 1. The results obtained with transfected COS-1 cells (fig. 9) were very similar to those found with human liver cytosol (fig. 1). There were two peaks of CST activity (fig. 9), and maximal activities were present at cholesterol concentrations of approximately 0.5 and 200 μM.

Discussion

Cholesterol is one of many endogenous compounds that undergo sulfation, but the nature of the enzyme or enzymes responsible for catalyzing the sulfate conjugation of cholesterol in human liver is unclear. We chose a combined biochemical and molecular approach to study cholesterol sulfation in human liver. Optimal conditions for the measurement of CST activity in human liver cytosol were determined, and we then compared the properties and regulation of human hepatic CST activity with those of human liver DHEA ST, TS PST, and TL PST. Two kinetic forms of CST activity with different apparent affinities for cholesterol were present in human liver preparations. The results of thermal inactivation and DCNP inhibition studies performed with hepatic cytosol preparations suggested that neither TS nor TL PST catalyzed the CST reactions, but the properties of DHEA ST were similar to those of both kinetic forms of human liver CST activity. The possibility that DHEA ST might catalyze the sulfation of cholesterol in human liver was also supported by the results of experiments performed with 20 individual human liver samples. Those data demonstrated that levels of DHEA ST activity were highly correlated with the activities of both high- and low-affinity CST, but not with the activities of either TS or TL PST. Therefore, the results of experiments performed with hepatic tissue preparations indicated that DHEA ST was the major enzyme capable of catalyzing the sulfate conjugation of cholesterol in human liver cytosol, although other enzymes may also be involved. Finally, we were able to test that hypothesis directly by expressing human liver DHEA ST cDNA in COS-1 cells (12). Those studies confirmed that DHEA ST could catalyze cholesterol sulfation, and that the biphasic substrate kinetics observed with human liver cytosolic preparations were also present in COS-1 cells that expressed human liver DHEA ST cDNA. Similar biphasic kinetic behavior has been observed with other drug-metabolizing enzymes in human liver (33). Although it is possible that posttranslational modification of DHEA ST might be responsible for this phenomenon, the molecular mechanism that underlies this kinetic behavior remains unclear and will have to be addressed in the course of future experiments.

Another intriguing puzzle is the enhancement of CST activity after ultracentrifugation that was reported originally in rabbit tracheal epithelium by Rearick *et al.* (5) and confirmed by our studies with human liver cytosol (table 1). Rearick *et al.* speculated that this behavior might result from the removal of endogenous cholesterol by centrifugation, but that explanation seems unlikely on the basis of our data for "no substrate" blanks (table 1). It is also possible that ultracentrifugation might remove a noncholesterol endogenous substrate that does not partition into the chloroform/methanol organic solvent used in this assay or that it might remove an endogenous enzyme inhibitor. Further experiments will also be required to address each of these possibilities in a systematic fashion. However, it is clear that human hepatic cytosol CST activity should be measured in HSS rather than 100,000*g* supernatant preparations.

The sulfation of cholesterol was previously studied primarily

in epithelial cells. It has been suggested that cholesterol sulfate might play a role in the differentiation of epithelial cells (5–7), and elevated epidermal cholesterol sulfate concentration is a hallmark of the dermatologic disease X-linked ichthyosis—a condition that results from an inherited deficiency of the sulfatase that hydrolyzes cholesterol 3-sulfate (3). However, the properties of the enzyme or enzymes that catalyze the sulfation of cholesterol in epithelial cells appear to differ from those of human liver CST. For example, CST activity in rabbit tracheal epithelia is activated by NaCl and Triton X-100 (5), but we found that human liver CST activity was inhibited by both NaCl and Triton X-100. Therefore, it cannot be assumed that CST activity in human tissues other than liver is catalyzed by DHEA ST nor can it be assumed that DHEA ST is the only enzyme in human liver cytosol that is capable of catalyzing this reaction—only that it appears to be the most important quantitatively. It should also be noted that CST and DHEA ST are differentially affected by NaCl, and the reasons for this discrepant behavior are not clear. All of these issues, among others, will be clarified by the molecular cloning of cDNAs for human ST enzymes other than DHEA ST. Finally, it should be emphasized that studies of the expression of cDNAs can be misleading if interpreted in isolation. Merely observing that expression of a cDNA results in the production of an enzyme capable of catalyzing the sulfation of a particular substrate does not eliminate the possibility that other enzymes might also be able to catalyze that reaction. In addition, such experiments give little indication of the relative *in vivo* importance of any particular enzyme. The approach that we took combines the strengths of experiments performed with tissue preparations with the strengths of experiments performed using the techniques of molecular biology. In this case, both approaches demonstrated the existence of high- and low-affinity kinetic forms of CST activity in human hepatic cytosol, that DHEA ST was the major enzyme responsible for catalyzing the sulfate conjugation of cholesterol in the human liver, and that DHEA ST catalyzed both the high- and low-affinity CST reactions in this important human drug-metabolizing organ.

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References

1. G. J. Mulder and W. B. Jakoby: Sulfation. In "Conjugation Reactions in Drug Metabolism" (G. J. Mulder, ed.), pp. 107–161. Taylor & Francis Ltd., London, 1990.
2. R. M. Weinshilboum: Sulfate conjugation of neurotransmitters and drugs: an introduction. *Fed. Proc.* **45**, 2220–2222 (1986).
3. L. J. Shapiro: Steroid sulfatase deficiency and X-linked ichthyosis. In "Metabolic Basis of Inherited Disease" (C. R. Scriver, A. L. Beaudet, W. S. Sly, and D. Valle, eds.), pp. 1945–1964. McGraw-Hill, Inc., New York, 1989.
4. M. A. Lampe, A. L. Burlingame, J. A. Whitney, M. L. Williams, B. E. Brown, E. Reitman, and P. M. Elias: Human stratum corneum lipids: characterization and regional variations. *J. Lipid Res.* **24**, 120–130 (1983).
5. J. I. Rearick, P. W. Albro, and A. M. Jetten: Increase in cholesterol sulfotransferase activity during *in vitro* squamous differentiation of rabbit tracheal epithelial cells and its inhibition by retinoic acid. *J. Biol. Chem.* **262**, 13069–13074 (1987).
6. A. M. Jetten, M. A. George, C. Nervi, L. R. Boone, and J. I. Rearick: Increased cholesterol sulfate and cholesterol sulfotransferase activity in relation to the multi-step process of differentiation in human epidermal keratinocytes. *J. Invest. Dermatol.* **92**, 203–209 (1989).
7. J. I. Rearick, T. W. Hesterberg, and A. M. Jetten: Human bronchial epithelial cells synthesize cholesterol sulfate during squamous differentiation *in vitro*. *J. Cell. Physiol.* **133**, 573–578 (1987).
8. G. Bleau, G. Lalumiere, A. Chapdelaine, and K. D. Roberts: Red cell surface structure stabilization by cholesterol sulfate as evidenced by scanning electron microscopy. *Biochim. Biophys. Acta* **375**, 220–223 (1975).
9. J. Langlais, M. Zollinger, L. Plante, A. Chapdelaine, G. Bleau, and K. D. Roberts: Localization of cholesteryl sulfate in human spermatozoa in support of a hypothesis for the mechanism of capacitation. *Proc. Natl. Acad. Sci. USA* **78**, 7266–7270 (1981).
10. G. A. Johnson, C. A. Baker, and K. A. Knight: Minoxidil sulfotransferase, a marker of human keratinocyte differentiation. *J. Invest. Dermatol.* **98**, 730–733 (1992).
11. C. N. Falany, M. E. Vazquez, and J. M. Kalb: Purification and characterization of human liver dehydroepiandrosterone sulphotransferase. *Biochem. J.* **260**, 641–646 (1989).
12. D. M. Otterness, E. D. Wieben, T. C. Wood, R. W. G. Watson, B. J. Madden, D. J. McCormick, and R. M. Weinshilboum: Human liver dehydroepiandrosterone sulfotransferase: molecular cloning and expression of cDNA. *Mol. Pharmacol.* **41**, 865–872 (1992).
13. A. Radominska, K. A. Comer, P. Zimniak, J. Falany, M. Iscan, and C. N. Falany: Human liver steroid sulphotransferase sulphates bile acids. *Biochem. J.* **272**, 597–604 (1990).
14. N. R. C. Campbell, J. A. Van Loon, and R. M. Weinshilboum: Human liver phenol sulfotransferase: assay conditions, biochemical properties and partial purification of isozymes of the thermostable form. *Biochem. Pharmacol.* **36**, 1435–1446 (1987).
15. C. N. Falany, M. E. Vazquez, J. A. Heroux, and J. A. Roth: Purification and characterization of human liver phenol-sulfating phenol sulfotransferase. *Archiv. Biochem. Biophys.* **278**, 312–318 (1990).
16. G. Rein, V. Glover, and M. Sandler: Phenolsulphotransferase in human tissue: evidence for multiple forms. In "Phenolsulfotransferase in Mental Health Research" (M. Sandler and E. Usdin, eds.), pp. 98–126. MacMillan Publishing Ltd., London, 1981.
17. G. Rein, V. Glover, and M. Sandler: Multiple forms of phenolsulfotransferase in human tissues: selective inhibition by dichloronitrophenol. *Biochem. Pharmacol.* **31**, 1893–1897 (1982).
18. C. Reiter and R. M. Weinshilboum: Acetaminophen and phenol: substrates for both a thermostable and a thermolabile form of human platelet phenol sulfotransferase. *J. Pharmacol. Exp. Ther.* **221**, 43–51 (1982).
19. C. Reiter, G. Mwaluko, J. Dunnette, J. Van Loon, and R. Weinshilboum: Thermolabile and thermostable human platelet phenol sulfotransferase: substrate specificity and physical separation. *Naunyn-Schmied. Arch. Pharmacol.* **324**, 140–147 (1983).
20. W. F. Young, Jr., H. Okazaki, E. R. Laws, Jr., and R. M. Weinshilboum: Human brain phenol sulfotransferase: biochemical properties and regional localization. *J. Neurochem.* **43**, 706–715 (1984).
21. R. S. Sundaram, C. Szumlanski, D. Otterness, J. A. Van Loon, and R. M. Weinshilboum: Human intestinal phenol sulfotransferase: assay conditions, activity levels and partial purification of the thermolabile (TL) form. *Drug Metab. Dispos.* **17**, 255–264 (1989).
22. J. S. Hernández, R. W. G. Watson, T. C. Wood, and R. M. Weinshilboum: Sulfation of estrone and 17β-estradiol in human liver: catalysis by thermostable phenol sulfotransferase and by dehydroepiandrosterone sulfotransferase. *Drug Metab. Dispos.* **21**, 413–422 (1992).
23. G. Powis, I. Jardine, R. Van Dyke, R. Weinshilboum, D. Moore, T. Wilke, W. Rhodes, R. Nelson, L. Benson, and C. Szumlanski: Foreign compound metabolism studies with human liver obtained as surgical waste; relation to donor characteristics and effects of tissue storage. *Drug Metab. Dispos.* **16**, 582–589 (1988).
24. A. Foldes and J. L. Meek: Rat brain phenolsulfotransferase—partial purification and some properties. *Biochim. Biophys. Acta* **327**, 365–374 (1973).
25. M. M. Bradford: A rapid and sensitive method for the quantitation

of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72,** 248–254 (1976).
26. G. G. Wong, J. S. Witek, P. A. Temple, K. M. Wilkens, A. C. Leary, D. P. Luxemberg, S. S. Jones, E. L. Brown, R. M. Kay, E. C. Orr, C. Shoemaker, D. W. Golde, R. J. Kaufman, R. M. Heurick, E. A. Wang, and S. C. Clark: Human GM-CSF: molecular cloning of the complementary DNA and purification of the natural and recombinant proteins. *Science* **228,** 810–815 (1985).
27. R. J. Kaufman: Identification of the components necessary for adenovirus translational control and their utilization in cDNA expression vectors. *Proc. Natl. Acad. Sci. USA* **82,** 689–693 (1985).
28. G. N. Wilkinson: Statistical estimations in enzyme kinetics. *Biochem. J.* **80,** 324–332 (1961).
29. W. W. Cleland: Computer programmes for processing enzyme kinetic data. *Nature* **198,** 463–465 (1963).
30. J. Langridge: Genetic and enzymatic experiments relating to the tertiary structure of β-galactosidase. *J. Bacteriol.* **96,** 1711–1717 (1968).
31. R. M. Weinshilboum: Enzyme thermal stability and population genetic studies: application to erythrocyte catechol-*O*-methyltransferase and plasma dopamine β-hydroxylase. In "Genetic Strategies in Psychobiology and Psychiatry" (E. S. Gershon, S. Matthysse, X. O. Breakefield, and R. D. Ciaranello, eds.), pp. 79–94. Boxwood Press, Pacific Grove, CA, 1981.
32. J. S. Hernández, S. P. Powers, and R. M. Weinshilboum: Human liver arylamine *N*-sulfotransferase activity: thermostable phenol sulfotransferase catalyzes the *N*-sulfation of 2-naphthylamine. *Drug Metab. Dispos.* **19,** 1071–1079 (1991).
33. T. A. Glauser, A. L. Kerremans, and R. M. Weinshilboum: Human hepatic microsomal thiol methyltransferase: assay conditions, biochemical properties and correlation studies. *Drug Metab. Dispos.* **20,** 247–255 (1992).