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Sulfotransferase Gene Expression in Primary Cultures of Rat Hepatocytes

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ABSTRACT. Hepatocyte cultures have been used in pharmacotoxicological studies, and sulfotransferases (ST) are important drug-metabolizing enzymes in liver. The expression of sulfotransferases in hepatocyte cultures has not been examined systematically. In the present study, the mRNA levels of different sulfotransferases in male and female rat hepatocytes were examined by northern-blot analyses. Various culture conditions such as different matrices (collagen, matrigel, collagen sandwich, or co-culture with epithelial cells), medium (Waymouth's MB 752/1 and Modified Chee's Medium) and glucocorticoid supplementation (dexamethasone, 0.1 μM) were compared. Phenol ST (ST1A1) mRNA levels decreased to about 50% of initial mRNA levels within 10 hr of culture. At 96 hr, ST1A1 mRNA levels were approximately 20% of initial values when cultured on collagen, matrigel or co-culture. The two media did not differ in ability to maintain ST1A1 mRNA levels in the absence of dexamethasone (DEX); however, DEX addition to either medium resulted in ST1A1 mRNA levels greater than 100% of the initial mRNA levels at 96 hr, with the greatest increase observed using the matrigel substratum and Chee's medium. In the absence of DEX, the mRNA levels of *N*-hydroxy-2-acetylaminofluorene sulfotransferase (ST1C1), estrogen sulfotransferase (ST1E2) and hydroxysteroid sulfotransferase (ST-20/21, ST-40/41, ST-60) fell to approximately 20% of their initial levels within 24 hr, and to less than 5% at 96 hr. The loss of expression of these sulfotransferases was observed with all culture conditions. Addition of DEX to the media resulted in ST-40/41 and ST-60 mRNA expression at 20 and 35% of their initial values, respectively, in cultures maintained on matrigel and Chee's medium at 96 hr. These data suggest that sulfotransferases lose their constitutive expression in hepatocyte culture, but retain their inducibility. BIOCHEM PHARMACOL 52;10:1621–1630, 1996. *Copyright © 1996 Elsevier Science Inc.*

KEY WORDS. sulfotransferase mRNAs; rat hepatocyte primary culture; phenol (ST1A1) mRNA; dexamethasone; matrigel; conditioned culture.

Primary hepatocyte culture has become an established method for pharmacotoxicological studies. It is also utilized to study xenobiotic biotransformation [1], drug-metabolizing enzyme regulation [2, 3], as well as for determining the mechanisms of enzyme regulation [4–7].

ST† are the class of conjugating enzymes that sulfate a wide range of endogenous and xenobiotic compounds. Sulfotransferases are regulated by hormones [8, 9] and steroidal chemicals [10]. To further study the regulation of sulfotransferases, primary hepatocyte culture might be a good model system. However, sulfotransferase expression in hepatocyte culture has not been examined systemically.

One known problem associated with hepatocyte culture is the loss of some liver specific enzymes [11], especially cytochromes P450. Many attempts have been made to maintain or restore hepatic function by supplementing exogenous soluble factors, improving cell polarity and providing cell-cell communication. Several culture systems have been shown to be superior to conventional culture methods in terms of maintaining albumin production or cytochromes P450 levels. These include using a more complex extracellular matrix, such as matrigel [12], a collagen sandwich configuration [13, 14] or co-culture with nonparenchymal rat liver cells [15–17]. Furthermore, various media [18] and hormone supplementation [19, 20] also have been shown to improve hepatocyte survival and liver-specific functions. Whether these modified culture systems have any effects on sulfotransferase expression is not known.

The present study was designed to investigate the expression of three male-dominant sulfotransferases (ST1A1, ST1C1 and ST1E2) in hepatocytes from male rats, and three female-dominant sulfotransferases (ST-20/21, ST-40/40 and ST-60) in hepatocytes from female rats under conventional hepatocyte culture system and three other modified culture systems. Effect of medium formulation and glucocorticoid supplementation on sulfotransferase expression

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† *Abbreviations:* ST, sulfotransferase; DEX, dexamethasone; *N*-OH-AAF, *N*-hydroxy-2-acetylaminofluorene.

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was also determined. In addition, the relationships among sulfotransferase expression, cell morphology and albumin expression were examined.

MATERIALS AND METHODS

Animals and Materials

Adult male and female Sprague–Dawley rats, 11–12 weeks of age, were obtained from Sasco Inc. (Omaha, NE) and maintained in an AAALAC accredited facility. The animals were kept under a 12-hr light/dark cycle and allowed free access to water and Laboratory Chow 5001 (Purina, St. Louis, MO).

Vitrogen was purchased from CELTRIX Pharmaceuticals Inc. (Santa Clara, CA). Matrigel was prepared from Engelbreth-Holm-Swarm tumor cells propagated in C57 BL/6J female mice as described elsewhere [12], and stored at −80°. Rat liver epithelial cells (clone-9, CRL-1439) were purchased from American Type Culture Collection (Rockville, MD), and maintained in Ham's F-12K. Collagenase, Waymouth MB 752/1 medium and modified Chee's medium (Chee's medium) were obtained from Gibco BRL Life Technologies (Grand Island, NY). Insulin was purchased from Collaborative Research (Bedford, MA).

RNAzol B was purchased from Tel-Test, Inc. (Friendswood, TX). tRNA (yeast), terminal deoxynucleotidyltransferase and DNA G-25 quick-spin columns were from Boehringer-Mannheim (Indianapolis, IN). Zeta-probe GT membrane was from Bio-Rad (Richmond, CA). Deoxyadenosine 5′-[α-^{32}P]triphosphate (6000 Ci/mmol) was obtained from Amersham Corp. (Arlington Heights, IL). The oligonucleotide probes for ST1A1, ST1C1, ST-20/21 and ST-60 were synthesized by the University of Kansas Medical Center (KUMC) Biotechnology Support Facility (Kansas City, KS), and the probes for ST1E2, ST-40/41, albumin and 18s rRNA were provided by Dr. Timo Buetler (KUMC, Kansas City, KS). Morpho carbodiimide (1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-*p*-toluenesulfonate), Percoll and other chemicals were obtained from the Sigma Chemical Co. (St. Louis, MO).

Culture Conditions and Dish Preparation

The mRNA expression of six sulfotransferases was examined as a function of time in rat hepatocytes cultured on one of four matrices (including co-culture), two media for each matrix, with or without DEX in each medium. Vitrogen (type I collagen) was diluted to 100 μg/mL with a solution of morpho carbodiimide (130 μg/mL) and applied to each dish in a volume of 2 mL [21]. Before seeding the cells, the aqueous solution in the dishes was aspirated and the dishes were rinsed with PBS. These dishes were used for cells cultured on a simple "collagen" and for cells maintained in "co-culture." The "matrigel" dishes were prepared as follows: matrigel was thawed and maintained at 4° prior to use. Matrigel (12 mg/mL, 200 μL) was then applied and evenly spread on the bottom of each dish with a rubber policeman [12]. To prepare the dishes for the collagen "sandwich," neutralized collagen was prepared by mixing vitrogen (3 mg/mL), 10 × PBS and 0.1 N NaOH (8:1:1, v:v:v, pH 7.2 to 7.4) on ice, as recommended by the supplier. The mixture (200 μL) was then spread over the bottom of the dishes with a rubber policeman. The prepared dishes were kept overnight in a humidified incubator with an atmosphere containing 5% CO_2 at 37° prior to use.

Waymouth's and Chee's medium were prepared according to the supplier's instructions. Thymidine (10 μg/mL) and arginine (168 μg/mL) were also added to Chee's medium at the time of preparation. All media were serum-free media supplemented with penicillin (100 U/mL), streptomycin (100 μg/mL), fungizone (250 μg/mL), and insulin (5 μg/mL) as the only hormone. Part of Waymouth and Chee's media was supplemented with DEX dissolved in DMSO, and the final concentration of DEX in the medium was 0.1 μM.

Hepatocyte Isolation and Cell Culture

Rats were anaesthetized with an intraperitoneal injection of pentobarbital (65 mg/kg). Hepatocyte isolation was carried out by a modification of the method of Seglen [22]. Briefly, liver was perfused via the portal vein with a Ca^{2+}- and Mg^{2+}-free perfusion buffer (gassed with carbogen), followed by collagenase (0.03%, w/v). The digested liver was excised, and cell suspensions were prepared in medium (either Waymouth's or Chee's). The cells were washed in medium, and then resuspended in 45% isotonic Percoll and centrifuged for 5 min at 150 g. After an additional wash, the cells were suspended in fresh medium, seeded at a density of 3 × 10^6 cells/dish in 3 mL of medium (2 × 10^6 cells/dish for co-culture), and allowed to attach for 3–4 hr in a humidified incubator. The media were then replaced with one of the specified media. For hepatocytes maintained in co-culture, the medium was replaced with 3 mL of fresh medium containing 1–2 × 10^6 epithelial cells. For sandwiched hepatocytes, medium was aspirated, and 250 μL of the neutralized vitrogen mixture (same as described previously) was applied to the top of each hepatocyte monolayer and allowed to gel at 37° in an incubator for 45 min before medium replacement. All cells were cultured at 37° in 95% relative humidity and an atmosphere containing 5% CO_2, with medium renewed daily. Cells were harvested at 10, 24 and 96 hr after seeding. Cell morphology was monitored by phase-contrast microscopy, and photomicrographs of 96-hr cultures were taken with a Nikon TMS inverted microscope.

RNA Isolation

At the time of cell harvest, the medium was aspirated from each dish, and cells were washed with PBS. Cells were lysed in RNAzol B by several passages through a Pasteur pipet. The total RNA from freshly isolated cells was similarly extracted by lysing fresh cell pellet with RNAzol B. The

cell lysate from 2 to 3 dishes was pooled for each RNA sample. Chloroform (0.1 vol. chloroform for 1 vol. lysate) was added and mixed vigorously, followed by centrifugation. RNA in the aqueous phase was precipitated with the same volume of isopropanol and washed with 75% ethanol. RNA pellets were dissolved in 0.25% SDS. RNA yield and purity were assessed by absorbance at 260 nm and A260/A280 ratio.

Northern-Blot Analysis of RNA

Twenty micrograms of total hepatocyte RNA was denatured and subjected to electrophoresis (1.2% agarose gel containing 2 M formaldehyde). Following electrophoresis, RNA was transferred onto a Zeta probe GT membrane in 10 × SSC buffer (1 × SSC = 0.15 M sodium chloride, 0.015 M sodium citrate).

Probes and Labeling for Northern Blot

The DNA oligonucleotide probe (20 mer) for ST1A1 (5′-CTTCACATGCACTAGCGGTG-3′) was complementary to the corresponding region (82–101) of the phenol sulfotransferase cDNA [23]. The probe for ST1C1 (5′-CACCTAGTGTGGAAGGTCTG-3′) was complementary to nucleotides 1050–1069 of *N*-OH-2AAF sulfotransferase cDNA [24]. The probe for ST1E2 (5′-GCACTCCAGGTCAGGTATTC-3′) corresponds to the complement of nucleotides 364–383 of the cDNA sequence for estrogen sulfotransferase [25]. The probe for ST-20/21 (5′-CCTTTCCTCATGAGGCCAGT-3′) corresponds to the complement of nucleotides 761–780 of the cDNA sequence published by Ogura *et al.* [26]. The probe for ST-40/41 (5′-TGTCTAATTCTC GCATAGAC-3′) complements the sequence (nucleotides 547–566) within hydroxysteroid sulfotransferase cDNA as described by Ogura *et al.* [27]. The probe for ST-60 (5′-TTTCTTCTCCAGGGC GATCT-3′) is complementary to the corresponding region (436–455) of another hydroxysteroid ST cDNA (Genbank accession No. D14989). The probes were tailed with [α-^{32}P]dATP (6000 Ci/mmol) by terminal deoxynucleotidyltransferase. The labeled probes were purified on DNA G-25 quick-spin columns.

Hybridization

For ST1A1, prehybridization (20 mM sodium phosphate, pH 7.0; 7% SDS; 5 × Denhardt's solution; 100 μg/mL salmon sperm DNA; and 125 μg/mL yeast tRNA) was conducted at 52° for at least 4 hr. The hybridization was similar to the prehybridization except that the solution contained 2 × 10^6 cpm/mL of [^{32}P]-labeled probe, and the hybridization time was 16–18 hr. For the other STs, albumin and 18s rRNA, prehybridization was similar to that for ST1A1, except the solution contained 20% formamide and the temperature was 46°. Hybridization was carried out overnight with a similar prehybridization solution except that it contained 1 × Denhardt's solution and a radio-labeled oligonucleotide probe. Posthybridization washing followed with appropriate stringency. Autoradiography was performed by exposing X-ray film (X-Omat, Kodak, Sigma) for an appropriate time (1–7 days). The autoradiograms were scanned with a densitometer (Molecular Dynamics, Sunnyvale, CA), and the density values of the sulfotransferases or albumin were normalized to that of the corresponding 18s rRNA.

The experiment was conducted three times with hepatocytes from three different male or female rats, and similar results were obtained. Therefore, the results from one representative experiment with 2–3 pooled dishes for each point are shown in the figures.

RESULTS

Cell Morphology at 96 hr

Cell integrity was judged by attachment to dishes, uniform cytoplasm, well-defined cell borders, and formation of bile canaliculi. The presence of intact cells was observed at 96 hr of culture under all culture conditions. However, pronounced morphological differences were observed among the various culture conditions employed (Fig. 1). On a simple collagen substratum, cells spread, flattened and reached confluence (Fig. 1A). In contrast, the cells on matrigel were spherical and clustered into discrete colonies (Fig. 1B). The cells maintained in a collagen sandwich did not spread and flatten as seen on simple collagen, nor did they form clusters as cells maintained on a substratum of matrigel. Instead, cells in collagen sandwiched tended to form continuous cords or trabeculae (Fig. 1C). The morphology of the cells under co-culture exhibited a flattened appearance, similar to the cells on the simple collagen substratum, but were prevented from completely spreading by the presence of the epithelial cells in the adjoining spaces (Fig. 1D). No difference in cell morphology was observed between hepatocytes from male and female rats under the same culture conditions (data not shown).

Expression of Albumin mRNA in Primary Cultures of Male and Female Rat Hepatocytes

Albumin mRNA expression in male rat hepatocytes cultured on collagen, matrigel or in co-culture declined to about 50% of the initial level after 10 hr of culture, and tended to recover by 24 hr (Fig. 2). At 96 hr of culture, cells expressed albumin mRNA in the range of 25–40%, 25–140%, and 25–60% of the initial levels on collagen, matrigel and co-culture, respectively, depending on the medium conditions. The hepatocytes cultured in Chee's medium had relatively higher albumin mRNA levels than the cells cultured in Waymouth's medium under most conditions. DEX supplementation of the media did not affect albumin mRNA expression in cells cultured on a collagen substratum, but hepatocytes cultured in matrigel and co-culture with DEX supplementation supported higher albumin mRNA expression. The cells cultured on matrigel in Chee's

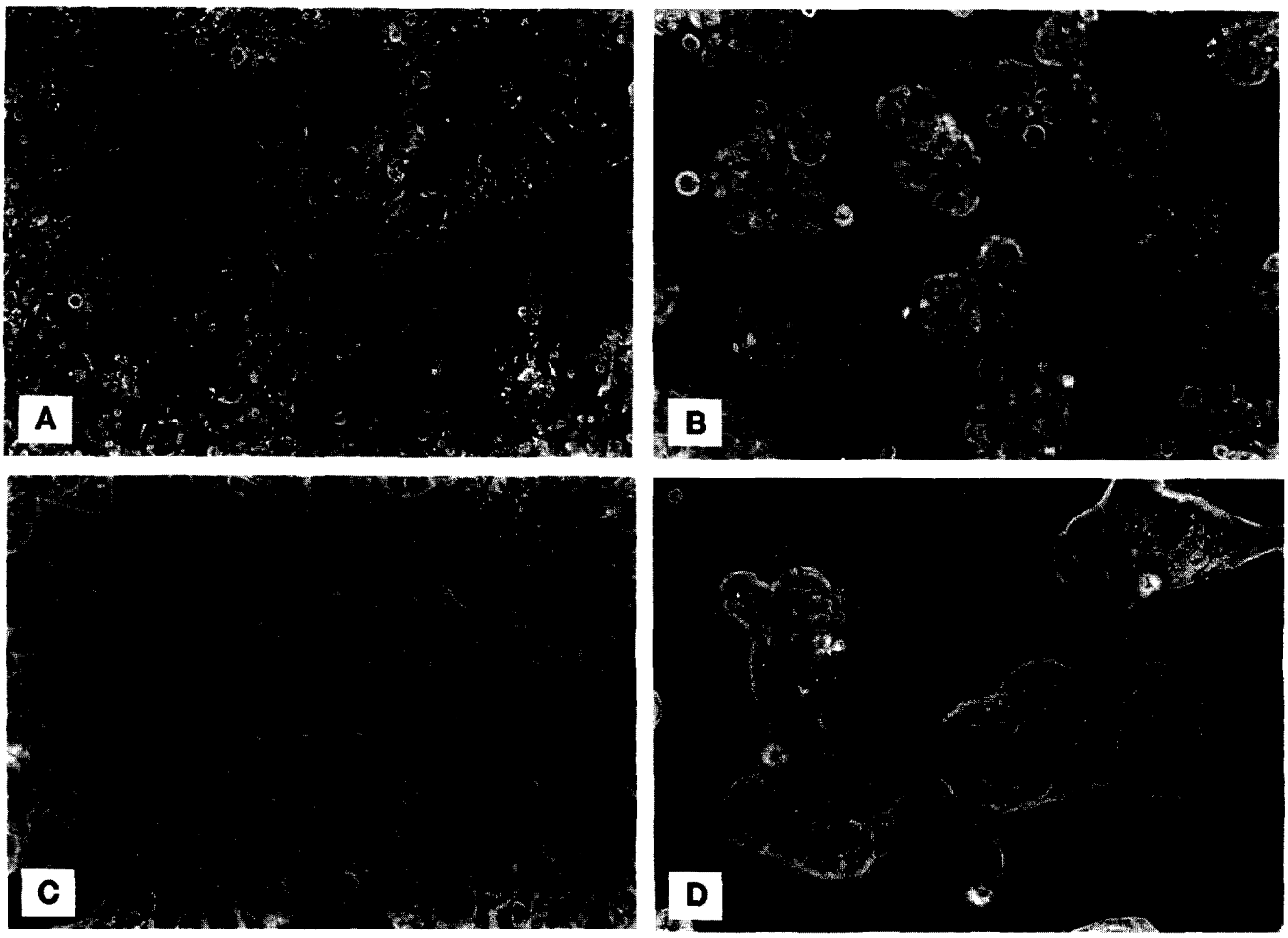

FIG. 1. Phase-contrast photomicrographs (200x) of hepatocytes on various matrices at 96 hr culture. A, simple collagen; B, matrigel; C, collagen sandwich; D, co-culture.

medium containing DEX expressed more albumin than freshly isolated cells. Surprisingly, cells maintained in a collagen sandwich exhibited the lowest expression of albumin mRNA. At 10 hr of culture, the albumin mRNA levels decreased about 80%, and declined further by 24 hr. At 96 hr of culture, albumin mRNA expression began to recover, but only reached approximately 10% of the initial mRNA levels.

The pattern of albumin mRNA expression in female rat hepatocytes was similar to that observed in male rat hepatocytes under the same culture conditions. However, female rat hepatocytes showed a greater response to DEX (data not presented). On collagen, matrigel or in co-culture, DEX in either media resulted in 100% or higher albumin expression in female rat hepatocytes at 96 hr. Even in the collagen sandwich, cells expressed about 35–60% of the initial albumin mRNA level when the medium contained DEX.

Expression of Phenol Sulfotransferase (ST1A1) mRNA in Primary Cultures of Male Rat Hepatocytes

The mRNA levels of ST1A1 in cells cultured under various culture conditions were examined at the indicated times (Fig. 3). All ST mRNA levels in freshly isolated hepatocytes were similar to the levels in liver tissue (data not shown). At 10 hr, ST1A1 mRNA levels had declined by approximately 30–50%, 40–65%, 70–80% and 30–65% on collagen, matrigel, collagen sandwich and co-culture, respectively. ST1A1 mRNA expression tended to recover by 24 hr of culture under all conditions, except with the collagen sandwich. At 96 hr of culture in the absence of DEX, the ST1A1 mRNA levels were approximately 15, 30, 10 and 25% of the initial mRNA levels on collagen, matrigel, sandwich and co-culture, respectively. There were no differences between ST1A1 mRNA levels from cells cultured with either Waymouth's or Chee's medium in the absence of DEX. At 96 hr of culture, DEX addition to the media resulted in ST1A1 mRNA levels greater than that from freshly isolated hepatocytes (>100%), except for the cells cultured in a collagen sandwich, which were increased only to 30%. When grown on matrigel or in co-culture in the presence of DEX, hepatocytes attained about 600 or 300% of initial ST1A1 expression level in Chee's medium. In Waymouth's medium, hepatocytes expressed 250 and 150% of initial ST1A1 mRNA levels, respectively. The cells cul-

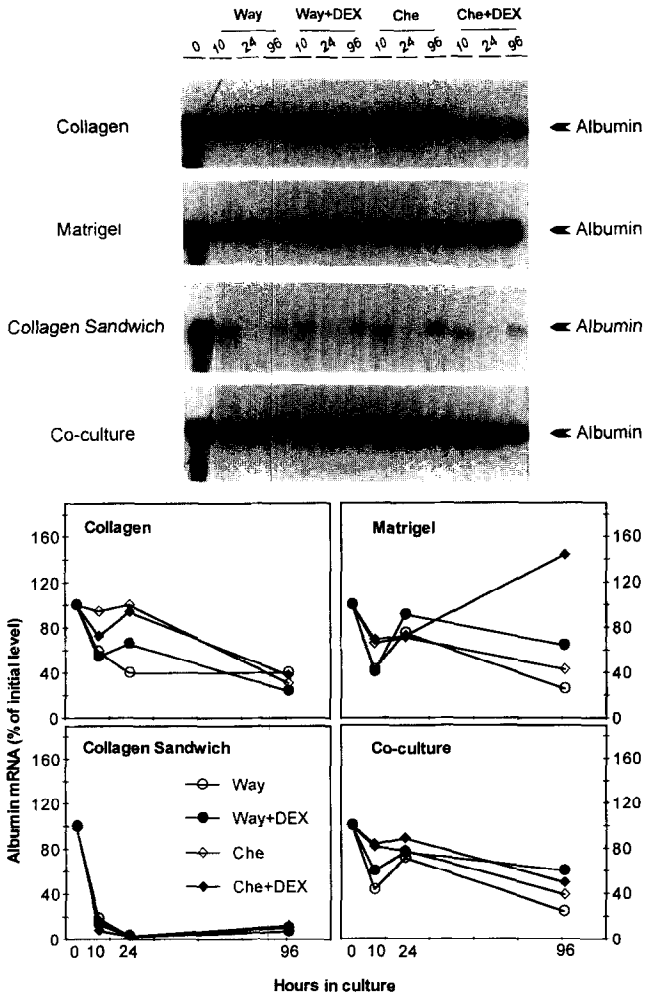

FIG. 2. Expression of albumin mRNA as a function of culture time in primary culture of male rat hepatocytes under various culture conditions. Cells were cultured on simple collagen (collagen), matrigel (matrigel), collagen sandwich, or co-cultured with liver epithelial cells (co-culture) on simple collagen substratum; in Waymouth's medium (Way) or Chee's medium (Che); with or without 0.1 μM dexamethasone (DEX) for 10, 24 and 96 hr. Top: Representative autoradiogram of Northern-blot analysis. Bottom: Graphic presentation of the relative mRNA levels. The values are obtained as described under Materials and Methods. The mRNA level from freshly isolated hepatocytes is designated as the initial value and presented as 100% at 0 hr. Data are expressed as percent of initial value.

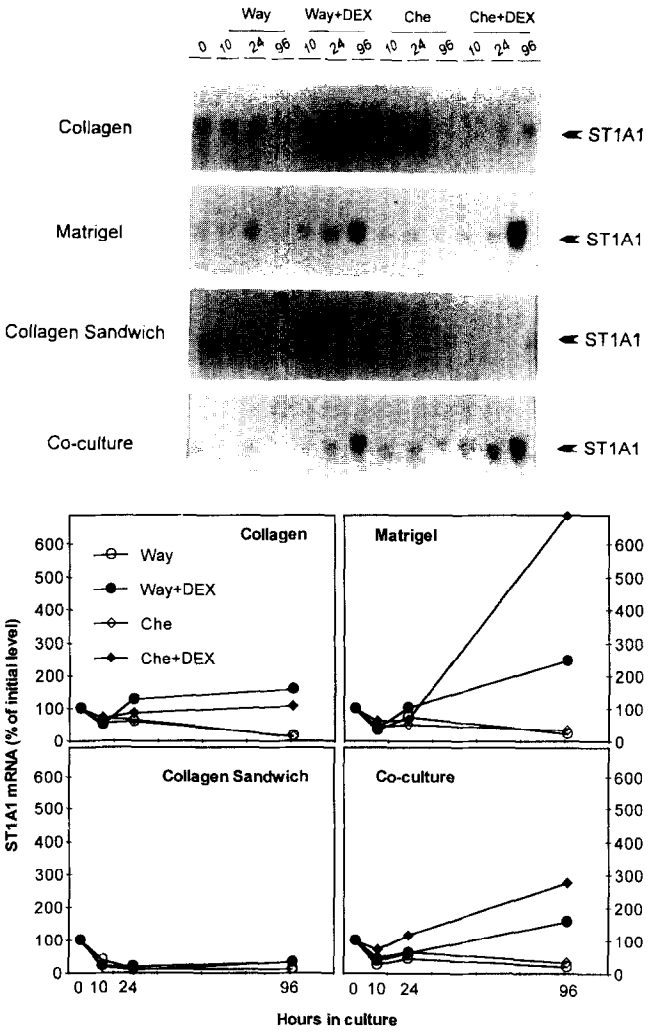

FIG. 3. Expression of ST1A1 mRNA as a function of culture time in primary culture of male rat hepatocytes under various culture conditions. See the legend to Fig. 2.

tured on matrigel in Chee's medium containing DEX yielded the highest mRNA levels, which were approximately 6-fold higher than the levels observed in fresh cells.

Expression of N-OH-2AAF Sulfotransferase (ST1C1) mRNA in Primary Cultures of Male Rat Hepatocytes

ST1C1 mRNA levels declined rapidly in cells cultured under all conditions (Fig. 4). At 10 hr of culture, the cells on collagen and in a collagen sandwich contained 40–70% and 10–40% of the initial mRNA levels, while the cells on

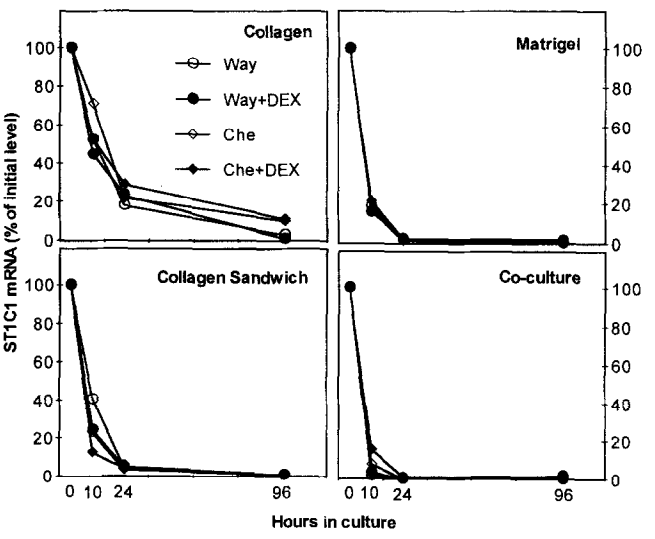

FIG. 4. Expression of ST1A1 mRNA as a function of culture time in primary culture of male rat hepatocytes under various culture conditions. See the legend to Fig. 2 (bottom panel).

matrigel and in co-culture expressed less than 20% of the initial mRNA levels. ST1C1 mRNA levels decreased further by 96 hr of culture. No difference in the ST1C1 mRNA levels was observed from cells cultured in either of the two media. The presence of DEX appeared to accelerate the decrease of ST1C1 mRNA levels during the 10 hr of culture when cells were cultured on collagen and collagen sandwich.

Expression of Estrogen Sulfotransferase (ST1E2) mRNA in Primary Cultures of Male Rat Hepatocytes

ST1E2 mRNA levels (Fig. 5) decayed rapidly as that seen for ST1C1. The decrease in ST1E2 mRNA levels was relatively slower in cells cultured on simple collagen than in those on other matrices. After 24 hr of culture, ST1E2 mRNA levels decreased to less than 5% of initial mRNA levels under all culture conditions tested and remained low thereafter.

Expression of Hydroxysteroid Sulfotransferase (ST-20/21, ST-40/41 and ST-60) mRNAs in Primary Culture of Female Rat Hepatocytes

Cells lost most of their ST-20/21 mRNA expression within the first 24 hr of culture (Fig. 6). The dramatic loss of expression of ST-20/21 was independent of matrices, media and DEX supplementation.

ST-40/41 mRNA expression also decreased rapidly in primary cultures of female rat hepatocytes (Fig. 7). At 10 hr, the cells cultured on collagen exhibited somewhat higher mRNA levels than cells cultured under other conditions. At 24 hr, there was no difference in the ST-40/41 mRNA levels observed in hepatocytes cultured under the various culture conditions tested. After 96 hr of culture,

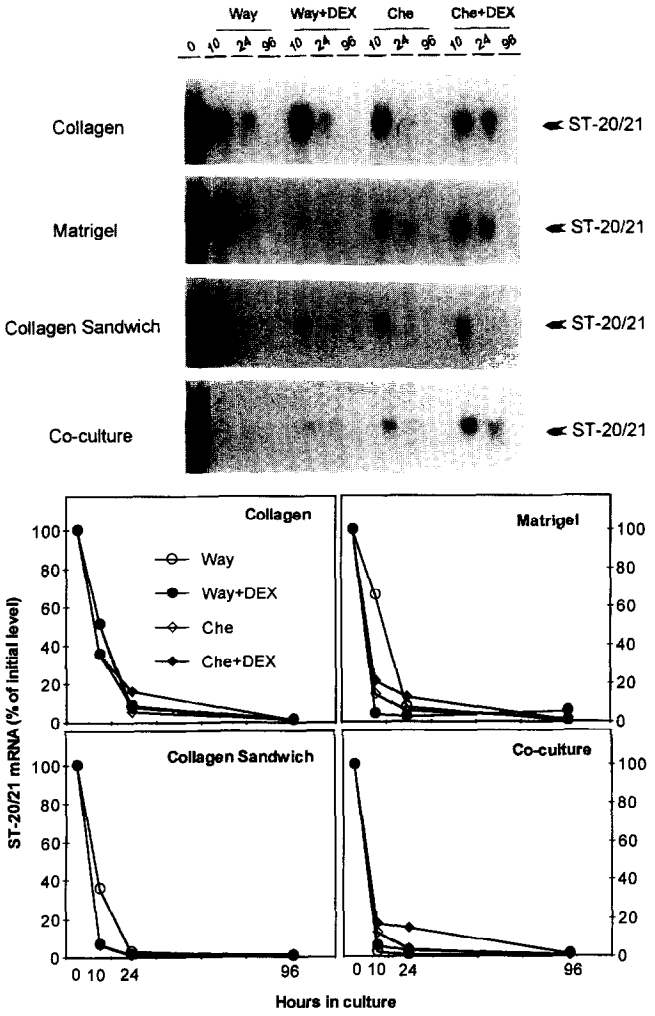

FIG. 6. Expression of ST-20/21 mRNA as a function of culture time in primary culture of female rat hepatocytes under various culture conditions. See the legend to Fig. 2.

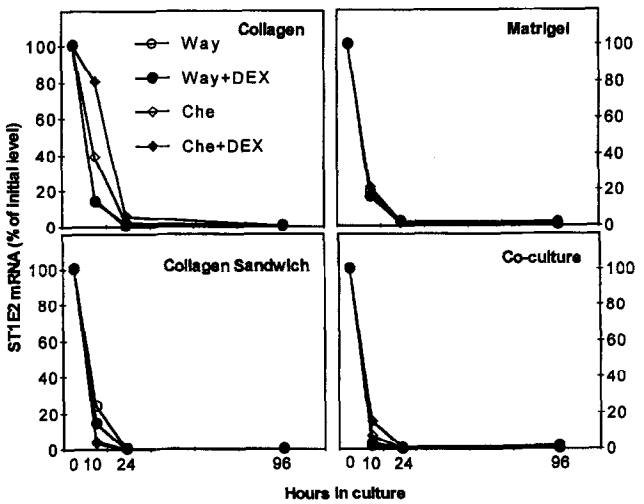

FIG. 5. Expression of ST1E2 mRNA as a function of culture time in primary culture of male rat hepatocytes under various culture conditions. See the legend to Fig. 2 (bottom panel).

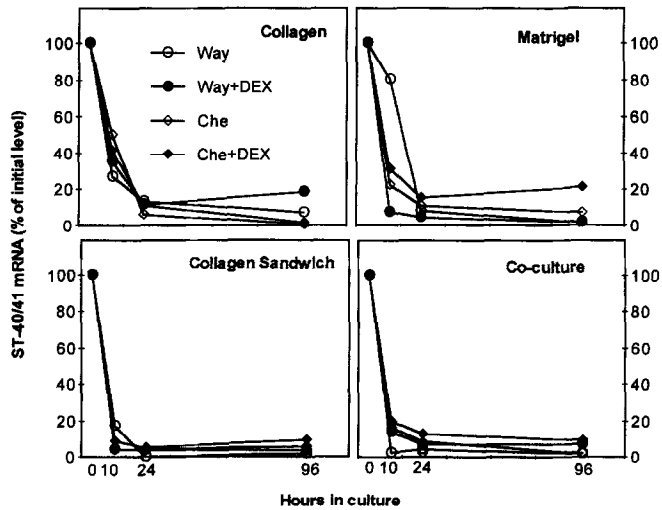

FIG. 7. Expression of ST-40/41 mRNA as a function of culture time in primary culture of female rat hepatocytes under various culture conditions. See the legend to Fig. 2 (bottom panel).

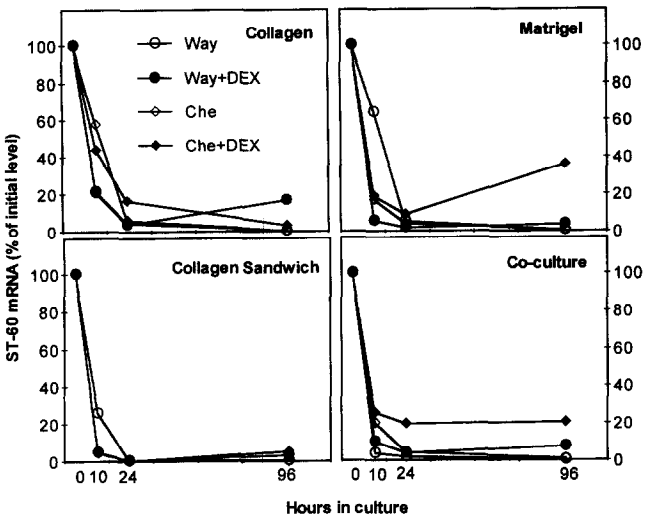

FIG. 8. Expression of ST-60 mRNA as a function of culture time in primary culture of female rat hepatocytes under various culture conditions. See the legend to Fig. 2 (bottom panel).

ST-40/41 mRNA began to increase in cells cultured on collagen or matrigel with medium containing DEX.

ST-60 mRNA declined in a similar manner to ST-20/21 and ST-40/41 within the first 24 hr (Fig. 8). But by 96 hr of culture, the ST-60 mRNA levels in cells cultured on collagen with Waymouth's medium containing DEX increased to about 15% of the initial value, compared to less than 5% of the initial value at 24 hr. When the cells were cultured on matrigel substratum with Chee's medium containing DEX, cells expressed ST-60 mRNA at approximately 35% of the level in freshly isolated cells, whereas cells cultured with other media contained ST-60 mRNA at <5% of the initial values at 96 hr. The co-culture with Chee's medium containing DEX was able to maintain the ST-60 mRNA expression at about 20% of initial mRNA levels from 10 hr through 96 hr of culture.

DISCUSSION

Sulfotransferases lose their constitutive expression in hepatocyte culture, as is seen for cytochromes P450. In a conventional hepatocyte culture system (simple collagen substratum and conventional medium with insulin as the only hormone), most sulfotransferase expression, except phenol sulfotransferase (ST1A1), showed rapid decline within the first 24 hr after hepatocyte isolation, and decreased further to less than 5% of initial expression levels at 96 of culture. The decrease of sulfotransferase mRNA levels might account for the decline in sulfotransferase activity [28], and the loss of the ability of hepatocytes to sulfate bile acids [29]. Albumin mRNA expression also decreased after hepatocyte isolation, but was restored at 24 hr. In general, albumin mRNA expression was much better maintained than the sulfotransferases in the same culture system. At 96 hr of culture, albumin mRNA expression was approximately 40% of initial values. The data indicate that hepatocytes preferentially lose drug metabolizing enzymes.

Hepatocytes cultured on matrigel, collagen sandwich and co-culture exhibit better morphology than cells cultured on simple collagen. The cluster and cord-like structure of hepatocytes on matrigel [12, 30] and in a collagen sandwich [13, 31] indicates a well conserved cell shape, which is associated with low cytoskeletal protein mRNA levels, as seen in freshly isolated hepatocytes. Addition of epithelial cells in culture provides enhanced cell-cell contact and extracellular matrix production, which in turn maintains cell architecture and function [32]. A common advantage of matrigel, collagen sandwich and co-culture over simple collagen are hepatocytes with maintained cell shape, polarity, and restored cell-cell communication.

Albumin secretion or albumin mRNA expression is often used to evaluate hepatocyte function. Several studies have reported that albumin secretion or mRNA expression is maintained at a higher level on matrigel [12], collagen sandwich [14] and co-cultures [16] than on a simple collagen substratum. In the present study, albumin mRNA expression of hepatocytes cultured on simple collagen was relatively high, whereas albumin mRNA levels in hepatocytes cultured in collagen sandwich were the lowest. The discrepancy between these results and those reported by Dunn *et al.* [14] might be due to the following reasons: (1) A different method of preparing simple collagen substratum was used in the present study (carbodiimide crosslinking [21]); whether this type of substratum or residual carbodiimide stimulates albumin expression is not known; (2) Different media and medium supplementation were employed in the present study. Although hepatocytes cultured in collagen sandwich expressed low levels of albumin, albumin mRNA expression levels began to increase at 96 hr of culture.

The expression of most sulfotransferases is not restored by factors improving cell structure, cell-matrix interaction and cell-cell communication. The decline in the expression of *N*-OH-2AAF sulfotransferase, estrogen sulfotransferase and hydroxysteroid sulfotransferase does not correspond to cell shape or recovery of albumin expression. This is also observed for some cytochromes P450. Although the co-culture system stabilizes CYP1A1 and 3A1 in hepatocyte cultures over conventional culture systems, CYP2B and CYP2C11 activities are still completely lost [33–35]. These results indicate that key factors, other than cell polarity, cell-matrix and cell-cell interaction, are missing in hepatocyte cultures.

Soluble factors might be very important for expression of sulfotransferases in hepatocyte culture. *In vivo*, the expression of most sulfotransferases and some cytochromes P450 shows dramatic sex differences, and is subject to pituitary hormone influence, especially growth hormone (GH) regulation [36]. For instance, *N*-OH-2AAF ST (ST1C1) and CYP2C11 are all stimulated by male GH secretory pattern [8, 37]. Expression of both enzymes rapidly falls during he-

patocyte culture (Fig. 4) [7, 28]. Addition of GH to culture medium indeed maintained CYP2C12 in female hepatocytes and induced CYP2C12 in male hepatocyte cultures [4, 5]. However, addition of GH for the first 24 hr to hepatocyte cultures did not prevent the loss of sulfotransferase expression (data not shown). GH in culture medium for three days did not alter sulfotransferase expression either [48]. Inasmuch as hepatocytes are exposed to a dynamic and complex environment of soluble factors *in vivo*, multiple hormone factors most likely are required for the expression of these sulfotransferases *in vitro*.

Phenol sulfotransferase (ST1A1) expression in hepatocyte culture is relatively better maintained than other sulfotransferases. In conventional hepatocyte culture systems, ST1A1 was also decreased at 10 hr of culture, but tended to be restored by 24 hr of culture. At 96 hr of culture, ST1A1 expression was 15% of initial values. Matrigel and co-culture resulted in increased expression (30 and 25%) of ST1A1 compared to the conventional collagen system (15%) at 96 hr of culture. Acetaminophen sulfation, which is catalyzed by a phenol sulfotransferase, has also been shown to be better maintained on matrigel than on simple collagen [39]. Using 2-naphthol as the substrate, phenol sulfotransferase activity decreased about 80% in hepatocytes cultured on collagen, while the sulfotransferase activity was maintained at about 50% in co-cultured hepatocytes [40]. *In vivo*, ST1A1 exhibited less sex difference than other sulfotransferases examined, and is not influenced by GH and steroid hormones [36]. This might be the reason that ST1A1 is better maintained in hepatocyte culture, and ST1A1 expression is improved by cell-matrix and cell-cell interaction.

DEX, a soluble factor, induces sulfotransferase expression in hepatocyte cultures. Glucocorticoids have been shown to improve the survival, function and morphology of hepatocytes in culture [41]. Low concentrations of DEX (10–100 nM) in the medium prevents deterioration and detachment of hepatocytes in long-term cultures. The intention of this study was to test whether DEX is an essential soluble factor to maintain sulfotransferase expression *in vitro*. The majority of hepatocyte culture systems contain 1 μM DEX [40, 42], or an equivalent amount of hydrocortisone [14, 16]. DEX at 1 μM has been shown to induce glucuronosyltransferase [3] and cytochrome P450 [2] in hepatocyte cultures. Phenol sulfotransferase activity was claimed to be maintained at initial levels for 7 days when cultured on collagen gel in the presence of 1 μM DEX and serum [40]. In contrast, McMillan *et al.* [28] observed an inhibitory effect of 1 μM DEX on phenol sulfotransferase and estrogen sulfotransferase activities in hepatocyte cultures. In order to minimize potential inducing or suppressing effects of DEX, a concentration of DEX one order of magnitude lower (0.1 μM) was used in this study. However, in the present study, 0.1 μM DEX increased ST1A1 expression over the initial mRNA levels, suggesting an inductive effect of DEX instead of a simple maintenance effect. In addition, ST-60 expression, which is less than 5% of initial values at 96 hr of culture in all culture systems, was increased to 15–35% of initial values in collagen, matrigel and co-culture systems by DEX. The induction of phenol and hydroxysteroid sulfotransferase mRNA by DEX was also reported by Runge-Morris *et al.* [38]. These data indicate that, like cytochrome P450, the constitutive expression of sulfotransferases is consistently lost in hepatocyte culture, but inducibility of the enzyme is maintained.

The inducibility of sulfotransferase by DEX is influenced by the matrix and medium conditions. For both ST1A1 and ST-60, the maximal induction by DEX was observed on the matrigel substratum and with Chee's medium. The higher enzyme induction of sulfotransferases on matrigel than that observed on the other substrata does not appear to be due to maintenance of cell shape and polarity, because collagen sandwich and co-culture also provide a more normal cell morphology and more enhanced cell contact than the simple collagen matrix. In contrast to the culture systems that contain type I collagen, matrigel contains laminin (60%), type IV collagen (30%) and heparan sulfate proteoglycan (3%) [43]. The presence of laminin in extracellular matrix has been shown to affect albumin and laminin B2 gene expression [44]. The interactions between biomolecules in matrigel and hepatocytes may not only result in modulation of hepatocyte gene expression, but may also alter the sensitivity to hormone induction. Certain amino acids, such as leucine, tryptophane, histidine and glutamine, inhibit protein degradation [45, 46], whereas alanine and pyruvate stimulate protein synthesis [47, 48]. Chee's medium contains higher levels of arginine, aspartate, leucine, serine, valine and glutamine, and has been shown to preserve cellular protein over prolonged culture periods [49]. Therefore, Chee's medium may provide an optimal environment for cells to respond to hormonal induction.

In summary, mRNA expression of most sulfotransferases such as *N*-OH-2AAF sulfotransferase (ST1C1), estrogen sulfotransferase (ST1E2) and hydroxysteroid sulfotransferase (ST-20/21, ST-40/41 and ST-60) are rapidly lost in primary cultures of rat hepatocytes. Various culture conditions of matrices and media did not prevent the loss of sulfotransferase expression. Complex and multiple soluble factors are apparently required for their expression *in vitro*. The inducibility of sulfotransferases is conserved and was dependent upon culture conditions, with the highest induction observed using the matrigel substratum and Chee's medium.

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