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SULPHOTRANSFERASE-MEDIATED ACTIVATION OF THE CARCINOGEN 5-HYDROXYMETHYL-CHRYSENE

SPECIES AND SEX DIFFERENCES IN TISSUE DISTRIBUTION OF THE ENZYME ACTIVITY AND A POSSIBLE PARTICIPATION OF HYDROXYSTEROID SULPHOTRANSFERASES

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Abstract—Sulphation of the carcinogen 5-hydroxymethyl-chrysene (5-HCR) to the active metabolite 5-HCR sulphate occurred at significant rates in all of hepatic cytosols prepared from the male and female experimental animals, rats, mice, guinea-pigs and hamsters. The 5-HCR-sulphating activity was also found in kidney cytosols of all the experimental animals used, while their activities were much less than those of hepatic cytosols. In the male mice, the enzyme activity of testis was higher than any other examined tissue. Small intestine and adrenal of male and female guinea-pigs had relatively high enzyme activities. Small enzyme activities were also found in a variety of extrahepatic tissues of some of these animals. Marked species and sex differences (female ≫ male in the rat and mouse) were observed in the hepatic enzyme activity. In the female rat liver which showed the highest 5-HCR-sulphating activity among the examined tissues of all the animals, a typical hydroxysteroid sulphotransferase inhibitor, dehydroepiandrosterone (DHA) sulphate (1 mM), potently and competitively inhibited the sulphation of 5-HCR as well as that of DHA, a typical substrate for hydroxysteroid sulphotransferases. On the contrary, the phenol sulphotransferase inhibitors, pentachlorophenol and 2,6-dichloro-4-nitrophenol, had only a little effect on these enzyme activities even at a concentration of 50 μM that showed a potent inhibition of the phenol sulphotransferase activity. These results suggest that 5-HCR be sulphated in the female rat liver by hydroxysteroid sulphotransferases, but not by phenol sulphotransferases.

5-Methylchrysene (5-MCR), an environmental carcinogen found in tobacco smoke, showed carcinogenicity to infant mouse liver and lung [1] as well as to adult mouse skin [2]. Metabolic oxidation of 5-MCR gave 5-hydroxymethyl-chrysene (5-HCR) as a major metabolite, dihydrodiols and phenols in the rat and mouse livers *in vitro* [3, 4] and in the mouse skin *in vivo* [4]. 5-HCR had as high a carcinogenicity to mouse skin as its parent compound, suggesting it to be one of the proximate metabolites in carcinogenesis induced by 5-MCR [5].

The carcinogen 5-HCR was demonstrated to induce His^+ reverse mutation in *Salmonella typhimurium* TA98 to a remarkable extent in the presence of rat liver cytosol fortified with a 3′-phosphoadenosine 5′-phosphosulphate (PAPS)-generating system [6]. From the PAPS-rat liver and mouse skin cytosol systems, the potently intrinsic mutagen 5-HCR sulphate was isolated as the sole metabolite and identified with a synthetic specimen [6, 7]. The active sulphate bound covalently for the most part to the exocyclic amino groups of the purine base residues (adenine ≫ guanine) of calf thymus DNA through its 5-methylene carbon with loss of a sulphate anion [8]. Similar sulphotransferase-mediated activation of hydroxymethyl-arenes has been also demonstrated with hydroxymethylbenz[*a*]anthracenes (−BAs) which were carcinogenic major metabolites of corresponding methyl-BAs [9–12]. However, except for the mouse skin, nothing is known of the sulphotransferase activity for the hydroxymethyl-arenes in extrahepatic tissues, because no sensitive and rapid method has been available for the assay of the enzyme activity sulphating these substrates. A promising approach to this problem could be to use 5-HCR as a substrate, for the ultimate metabolite, 5-HCR sulphate, is the most stable ($T_{1/2}$ = 11 hr at pH 7.4 and 37°) among the known sulphate esters formed from the carcinogenic hydroxymethyl-arenes [13].

This paper deals with (i) rapid and sensitive assay of sulphotransferase activity by using radioactive 5-HCR; (ii) species and sex differences in tissue distribution of the enzyme activity; and (iii) effect of sulphotransferase inhibitors on 5-HCR-sulphating activity in rat liver to obtain indirect evidence for a possible participation of hydroxysteroid sulphotransferases in activation of 5-HCR.

MATERIALS AND METHODS

Materials. Dehydroepiandrosterone (DHA) was purchased from Wako Pure Chemical Industries, Ltd (Osaka, Japan), [1,2,6,7-^{3}H]DHA ([^{3}H]DHA, 97.3 Ci/mmol) from New England Nuclear (Boston, MA), and 4-nitrophenol (4-NP), sodium perchlorate, and tetra-*n*-butylammonium (TBA) bromide from Kanto Chemical Co. Inc. (Tokyo, Japan). 5-Formylchrysene [14], 5-HCR [15], and DHA sulphate (DHAS) [16] were prepared as previously

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reported. PAPS was chemically synthesized as a specimen free from inorganic salts and other nucleotides by the previously reported method [17]. The radioactive steroid was diluted with unlabelled DHA and purified to homogeneity with radiochemical purity of higher than 99% before use by high pressure liquid chromatography (HPLC) on a reverse partition column (μBONDAPAK C_{18}, 10 μm in particle size, 4.6 × 250 mm) in MeOH–H_2O (7:3, 1 ml/min); the steroid detected with a refracto-monitor (Japan Analytical Industry model RI-II) was eluted at 15 min. Homogeneity of the synthetic specimens was confirmed by HPLC and thin-layer chromatography. Other reagents used were of reagent grade.

Synthesis of [methylene-^{3}H]5-HCR ([^{3}H]5-HCR). [^{3}H]5-HCR was prepared by the reduction of 5-formylchrysene (14.1 mg) with [^{3}H]sodium borohydride (0.39 mg, 100 mCi, 10 Ci/mmol, Amersham International (Bucks, U.K.)) in 0.4% aqueous methanol (8.5 ml) containing 2 mmol of sodium hydroxide at 20° for 24 hr. After evaporation of the solvent from the reaction mixture under a nitrogen stream, 1 N hydrochloric acid (5 ml) was added to the residue. The aqueous acidic suspension of the residue was shaken three times with ethyl acetate (5 ml each), and the combined organic layer, containing [^{3}H]5-HCR and unreacted 5-formylchrysene, was dried over anhydrous sodium sulphate and condensed to dryness under the nitrogen stream. The residue obtained was chromatographed on a silica column (2 × 20 cm) by successive elution with chloroform-*n*-hexane (2:1, 100 ml) and chloroform (100 ml). The chloroform eluate was condensed to dryness under reduced pressure to give colourless needles. [^{3}H]5-HCR isolated in 90% radiochemical yield had a specific radioactivity of 2.1 Ci/mmol and a radiochemical purity higher than 99.9%, determined by the scintillation counting method and absorptiometry at 269 nm.

Preparation of dialysed cytosols of various tissues. Cytosols were prepared from the following male and female animals: six Wistar rats, weighing 190–200 g (7 weeks of age), twenty ddy mice, weighing 25–30 g (5 weeks of age), six Gordon Hartley guinea-pigs, weighing 250–300 g (7 weeks of age), and six golden Syrian hamsters, weighing 80–90 g (7 weeks of age), which were purchased from Tokyo Laboratory Animals Science Co. Ltd (Tokyo, Japan). Preparation of the cytosols was carried out at 0–4° as follows: the animals were anaesthetized with ether and decapitated, and their tissues were immediately removed and pooled in isotonic KCl chilled on an ice bath. Small intestine was pre-homogenized in isotonic KCl with a Polytron homogenizer (Kinematica, Luzern, Switzerland) after it was excised with scissors to obtain a 20–30 cm long portion of an oral end of jejunum. The pre-homogenized small intestine and other tissues were homogenized in isotonic KCl with a Teflon-pestled Potter–Elvehjem type of homogenizer. Cytosol fractions were obtained by centrifugation (105,000 *g*, 60 min) of post-mitochondrial fractions of 33% homogenates of livers, 25% homogenates of kidneys, lungs, testes and small intestines, and 16% homogenates of adrenals and ovaries. The supernatant fractions were dialysed at 0° for 24 hr against 2500 volumes of 0.1 M phosphate buffer, pH 7.4. Protein contents in the dialysed soluble supernatant fractions were determined by the method of Lowry *et al.* [18].

Assay of sulphotransferase activity towards 5-HCR. [^{3}H]5-HCR (2 μCi, 0.1 μmol) dissolved in dimethyl sulphoxide (DMSO, 0.1 ml) was incubated with cytosols (2 mg protein) fortified with PAPS (0.6 μmol) in 0.1 M phosphate buffer, pH 7.4, (0.9 ml) at 37° for 60 min. After addition of an aqueous solution (0.5 ml) of TBA bromide (6 mM) to the incubation mixture, [^{3}H]5-HCR and a hydrophobic ion-pair complex of metabolically formed [^{3}H]5-HCR sulphate with a TBA cation were extracted twice with *n*-hexane–ethyl acetate (1:1, 1.5 ml each) from the mixture. From the combined organic phase, [^{3}H]5-HCR sulphate was extracted as a sodium salt twice with an aqueous solution (1.5 ml each) of 5% sodium carbonate containing sodium perchlorate (1 M). The combined aqueous phase containing sodium [^{3}H]5-HCR sulphate was washed twice with *n*-hexane–ethyl acetate (2:1, 2 ml each) to remove a trace amount of the contaminant [^{3}H]5-HCR, and the radioactivity of the residual aqueous solution was determined in a dioxan scintillator by the liquid scintillation counting method. A calibration curve for the extracted sulphate ester, prepared by using sodium [^{3}H]5-HCR sulphate (2 pmol–20 nmol (5–10 nCi)/ml) and the various tissue cytosols (2 mg protein/ml), showed a good linearity with the radioactive sulphate ester added. The recovery of the radioactive sulphate ester from the incubation mixtures was 81–83% under the above conditions.

Assay of phenol and hydroxysteroid sulphotransferase activities. For determination of the phenol sulphotransferase activity in hepatic cytosols, 4-NP (0.1 μmol) dissolved in DMSO (0.1 ml) was incubated with the cytosols (2 mg protein) fortified with PAPS (0.12 μmol) in 0.1 M phosphate buffer, pH 7.4, (0.9 ml) at 37° for 30 min. 4-NP sulphate formed was determined as previously reported after addition of the Methylene blue reagent to the incubation mixture [19]. The hydroxysteroid sulphotransferase activity was assayed by using [^{3}H]DHA as a substrate as follows: [^{3}H]DHA (0.1 μCi, 5 nmol) dissolved in DMSO (0.1 ml) was incubated with hepatic cytosols (0.1 mg protein) fortified with PAPS (0.12 μmol) in 0.1 M phosphate buffer, pH 7.4, (0.9 ml) at 37° for 10 min. The incubation mixture was washed five times with *n*-hexane–ethyl acetate (1:1, 2 ml each) to remove the unreacted radioactive substrate. The radioactivity of the residual aqueous solution containing metabolically formed [^{3}H]DHA sulphate was determined in the dioxan scintillator by the liquid scintillation counting method.

Radioactivity counting. An Aloka model 903 liquid scintillation counter was used for counting radioactivities of sulphate esters of [^{3}H]5-HCR and [^{3}H]DHA.

RESULTS

Species and sex differences in tissue distribution of sulphotransferase activity towards 5-HCR

The ion-pair extraction method carried out by using TBA bromide as a counter cation donor was found to be useful for the rapid and sensitive assay of

Table 1. Species and sex differences in hepatic sulphotransferase activities towards 5-HCR, 4-NP, and DHA

Species	Sex	Formation of sulphate esters (pmol/mg protein/min)*		
		5-HCR	4-NP	DHA
Rat	M	40 (0.24)	197 (2.3)	957 (0.51)
	F	168	85	1885
Mouse	M	3.7 (0.22)	141 (0.82)	0.46 (0.20)
	F	17	173	2.27
Guinea-pig	M	76 (0.89)	432 (1.25)	318 (0.89)
	F	85	346	356
Hamster	M	69 (0.82)	59 (1.48)	497 (1.15)
	F	84	40	432

* Data are arithmetic mean values of at least four experiments. Numerals in parentheses represent ratios of male to female activities.

the enzyme (sulphotransferase) activities to sulphate the carcinogen 5-HCR to the potent mutagen 5-HCR sulphate in various tissues of experimental animal. The radioactive sulphate ester formed enzymically from [^{3}H]5-HCR was efficiently extracted as a hydrophobic ion-pair complex with the TBA cation into the organic solvent after the addition of TBA bromide to the cytosolic incubation mixtures and then separated without decomposition from the unreacted substrate by dissociation into a sodium salt with an aqueous solution of sodium perchlorate and carbonate.

Species and sex differences in hepatic sulphotransferase activity towards 5-HCR were compared with those in the enzyme activities towards DHA, a typical substrate for hydroxysteroid sulphotransferases, and 4-NP, a typical substrate for phenol sulphotransferases (Table 1). The hepatic cytosolic fractions of rats, hamsters and guinea-pigs showed potent sulphating activities towards 5-HCR, 4-NP, and DHA both in male and female. Mice showed much smaller hepatic enzyme activities towards 5-HCR and DHA than those of the above animals while the mouse liver 4-NP-sulphating activities were relatively high. The hepatic 5-HCR-sulphating activity was higher in the female than in the male of all species of the examined animals. In the rat and mouse, a marked sex difference (female $\gg$ male) was observed in the hepatic enzyme activity sulphating DHA as well as 5-HCR while only a little sex difference existed in the hepatic enzyme activities of the hamster and guinea-pig. On the contrary, except the mouse, the hepatic 4-NP-sulphating activity was higher in the male than in the female of these experimental animals.

A kinetic study of the enzyme reaction, carried out at concentrations ranging from 10 to 100 μM 5-HCR and 0.6 mM PAPS vs reaction rates for the formation of 5-HCR sulphate in the zero-order kinetic region, indicated that the significant sex difference in enzyme activity of the rat liver cytosols was attributable to the marked difference both in affinity of the substrate to the cytosolic sulphotransferases and in their catalytic activities; apparent K_m and V_{max} values were 114 μM and 148 pmol/mg protein/min for the male and 50 μM and 330 pmol/mg protein/min for the female.

Extrahepatic tissue distribution of the 5-HCR-sulphating activity was examined with kidney, lung, small intestine, adrenal, and testis (ovary) of the male (female) animals (Table 2). These tissue cytosols examined were prepared from the same animals as those used for the preparation of the hepatic cytosols. Except male rats and male and female hamsters, the 5-HCR-sulphating activity was present in all of the examined extrahepatic tissues of the other male and female animals. However, except testis of mice and small intestine and adrenal of male and female guinea-pigs, the enzyme activities in many of their extrahepatic tissues were less than a few per cent of their livers. Kidneys of all the male and female animals had the 5-HCR-sulphating activities (female > male). The enzyme activity was undetectable in the other tissues of the male rat and hamster. In the male mouse, however, testis showed higher enzyme activity than the other tissues including liver. Adrenal and small intestine of the guinea-pig were also found to have relatively high 5-HCR-sulphating activities.

Inhibition of 5-HCR sulphate formation by sulphotransferase inhibitors

Effects of sulphotransferase inhibitors on the sulphation of 5-HCR in the female rat liver cytosol with the highest specific enzyme activity were compared with those of the inhibitors on sulphation of 4-NP and DHA to obtain indirect evidence for the tentative classification of the 5-HCR-sulphating enzyme(s) to either hydroxysteroid sulphotransferase(s) or phenol sulphotransferase(s) (Table 3). DHAS (1 mM), a typical and competitive inhibitor for hydroxysteroid sulphotransferases, markedly inhibited sulphation of both 5-HCR and DHA while pentachlorophenol (PCP) and 2,6-dichloro-4-nitrophenol (DCNP), typical inhibitors for phenol sulphotransferases, had only a little effect on these enzyme reactions even at a concentration of 50 μM that showed potent inhibition of the 4-NP-sulphating activity. DHAS (1 mM), however, had only a little inhibitory effect on the 4-NP-sulphating activity. 3′-Phosphoadenosine 5′-phosphate (PAP), a cofactor analog to PAPS and a nonspecific inhibitor, inhibited the sulphation of all of these substrates. The enzyme activity of 5-HCR sulphation in the female rat liver cytosol was com-

Table 2. Species and sex differences in extrahepatic tissue distribution of sulphotransferase activity towards 5-HCR

Species	Sex	Formation of 5-HCR sulphate (pmol/mg protein/min)*					
		Kidney	Lung	Small intestine	Adrenal	Testis	Ovary
Rat	M	0.03	nd†	nd	nd	nd	—
	F	0.03	0.06	0.53	0.12	—	0.11
Mouse	M	0.09	0.06	0.04	0.09	5.06	—
	F	0.17	0.08	0.14	0.16	—	0.12
Guinea-pig	M	0.15	0.11	5.06	50.1	0.10	—
	F	0.72	0.24	6.28	9.5	—	0.28
Hamster	M	0.58	nd	nd	nd	nd	—
	F	0.90	nd	0.16	nd	—	nd

* Data are arithmetic mean values of at least four experiments.
† nd ≤ 0.01.

Table 3. Effect of sulphotransferase inhibitors on formation of sulphate esters of 5-HCR, 4-NP, and DHA in female rat liver cytosol

Inhibitor	Concentration (μM)	Formation of sulphate esters (pmol/mg protein/min)*		
		5-HCR	4-NP†	DHA
None	—	175 (0)	88 (0)	1930 (0)
PCP	10	173 (1)	39 (56)	1700 (12)
	50	164 (6)	24 (73)	1490 (23)
	100	158 (10)	21 (76)	1480 (23)
DCNP	10	165 (6)	44 (50)	1910 (1)
	50	164 (6)	32 (64)	1905 (1)
	100	159 (9)	24 (73)	1785 (8)
DHAS	100	150 (14)	86 (2)	1690 (12)
	500	92 (47)	78 (11)	1210 (37)
	1000	71 (59)	69 (22)	860 (55)
PAP	50	87 (50)	50 (43)	185 (90)
	100	62 (65)	38 (57)	45 (98)
	500	18 (90)	15 (83)	27 (99)

* Data are arithmetic mean values of at least four experiments. Numerals in parentheses represent per cent inhibition.
† Formation of 4-NP sulphate in the presence of DHAS was measured as previously reported [35].

petitively inhibited by DHAS at an apparent K_i of 190 μM (Fig. 1).

DISCUSSION

In the previous paper, the authors demonstrated that 7,12-dihydroxymethyl-BA (DHBA), a carcinogenic major metabolite of 7,12-dimethyl-BA (DMBA), was sulphated to give the potent mutagen, DHBA 7-sulphate, regiospecifically in the rat liver cytosol fortified with PAPS [11]. DHBA 7-sulphate was a highly reactive metabolite with a half-life of 3.5 min in water and bound to the exocyclic amino groups of the purine base residues (guanine > adenine) of calf thymus DNA [13]. The metabolic formation of the 7-sulphate ester from DHBA and its DNA binding were strongly inhibited by the hydroxysteroid sulphotransferase inhibitor DHAS while they were slightly inhibited by the phenol sulphotransferase inhibitors, PCP and DCNP [13]. A series of other carcinogenic hydroxymethyl-BAs, such as 7-hydroxymethyl-BA, 7-hydroxymethyl-12-methyl-BA, and 12-hydroxymethyl-7-methyl-BA, were also sulphated by rat liver cytosolic sulphotransferases to give the corresponding reactive sulphate esters with potent mutagenicity [9, 10, 12]. However, these sulphate esters have half-lives of more or less than a minute in water at pH 7.4 and are decomposed with formation of the parent alcohols [13], so that they cannot be used as substrates for the survey of tissue distribution of the hydroxymethyl-arene-sulphating activity determined by the isolation of the enzymic reaction products.

Unlike the sulphate esters of the hydroxymethyl-BAs, 5-HCR sulphate formed from the carcinogen 5-HCR by sulphotransferases has a half-life of 11 hr at pH 7.4 and 37° in water [13] and could be quantitatively extracted with *n*-hexane-ethyl acetate from the incubation mixtures without decomposition by using TBA bromide as a counter cation donor. The hydrophobic 5-HCR sulphate–TBA complex, extracted together with unreacted 5-HCR, was more stable in the aprotic solvent and could be readily dissociated and transferred into the aqueous alkaline solution, containing sodium perchlorate and carbonate, without any appreciable decomposition by the simple shaking. By this method, [^{3}H]5-HCR was separated from the water-soluble metabolite [^{3}H]5-HCR sulphate. It should be avoided to use the ethyl acetate extraction method for the direct removal of the unreacted [^{3}H]5-HCR from the incubation mixtures without TBA bromide, because recovery of [^{3}H]5-HCR sulphate, extracted as the ion-pair complex after that by the addition of TBA bromide, was markedly decreased by coagulation of cytosolic proteins. Furthermore, a small amount of [^{3}H]5-HCR stuck to the proteins could not be completely washed out of them by repeated extractions with organic solvents. Therefore, the simple method, removal of the unreacted substrate with organic solvents and subsequent radioactivity counting of the residual aqueous phase containing the sulphate, was

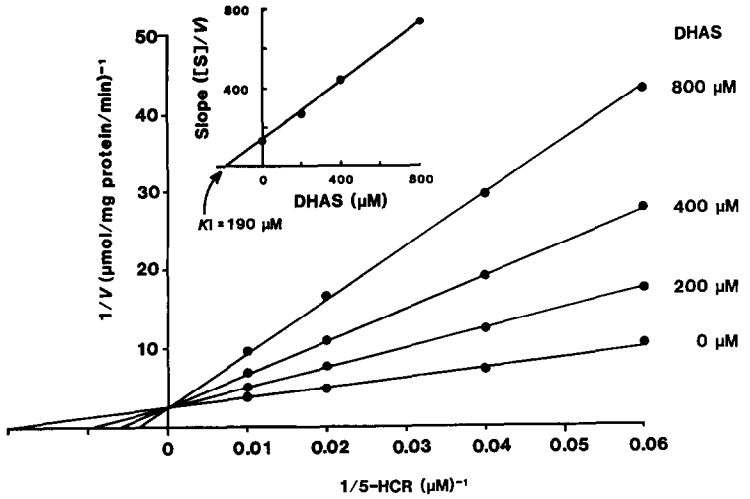

Fig. 1. Inhibitory effect of DHAS on the rate of formation of 5-HCR sulphate at various concentrations of 5-HCR in female rat liver cytosol. Sulphotransferase activities towards 5-HCR were measured in the presence and in the absence of DHAS by the method as described in the text and expressed as arithmetic mean values of four experiments. The figure at the bottom shows double reciprocal plots of the rates of 5-HCR sulphate formation vs concentrations of 5-HCR. The figure at the top shows a plot of concentrations of DHAS vs the slopes of the lines in the lower figure.

not applicable to the determination of small enzyme activities of the extrahepatic tissues. The quarternary ammonium cation, TBA ion, arising from its bromide added to the mixtures after the incubation, may play a role in preventing the strong anion, 5-HCR sulphate, from associating with cationic sites of the soluble proteins. Thus, a rapid and highly sensitive method was established in the present study by using [^{3}H]5-HCR for the exact determination of the sulphating activities of the carcinogenic hydroxymethylarenes in various tissues of experimental animals.

The most extensive works on sulphotransferases have been done with the rat liver cytosol [20, 21]. At least three types of sulphotransferases catalysing the sulphate conjugation of xenobiotics are known to exist in the rat liver cytosol [22]: phenol sulphotransferases for phenols and *N*-hydroxy-2-acetylaminofluorene (*N*-OH-AAF) [23], hydroxysteroid sulphotransferases for alcohols and steroids [24, 25], and amine sulphotransferases for aromatic primary and secondary amines [26, 27]. Rat liver hydroxysteroid sulphotransferases catalyse the sulphation of the 3β-hydroxysteroid, DHA, as a common substrate, and their activities are higher in the female animals than in the male [24, 25]. In addition, hydroxysteroid sulphotransferases are inhibited by the enzyme reaction product, DHAS [24, 25], although they not only are merely slightly inhibited by the phenol sulphotransferase inhibitors, PCP and DCNP [28], but also have no conjugation activity towards 4-NP, a typical substrate for phenol sulphotransferases [20, 21]. The sulphate conjugation of 5-HCR took place at a higher rate in the female rats than in the male animals and was competitively inhibited by DHAS in the female rat liver cytosol. Moreover, the enzymic conjugation reaction for 5-HCR was only slightly inhibited by the aforementioned phenol sulphotransferase inhibitors, whereas under the same conditions, they inhibited the sulphate conjugation of 4-NP strongly. These facts, therefore, indicate that at least in the female rat liver cytosol, 5-HCR be sulphated by hydroxysteroid sulphotransferases, but not by phenol sulphotransferases. In the mouse, hydroxysteroid sulphotransferase activities for DHA are known to be higher in the female than in the male as shown in the present study [29]. 5-HCR was also conjugated at a higher rate in the female mice than in the male animals.

It is of interest that 4-nitrobenzyl alcohol and methyl naphthyl carbinol have very recently been reported to undergo sulphation by rat liver cytosolic phenol sulphotransferase IV [30]. The enzyme would also have a high affinity to the small-sized aryl- or aralkyl-methanols other than phenols and *N*-OH-AAF.

5-HCR, a major carcinogenic metabolite of the potent carcinogen, 5-MCR, in untreated rat and mouse liver microsomes, does not induce carcinoma in their livers. The authors have demonstrated that in the liver, the reactive sulphate ester formed from 5-HCR is rapidly scavenged by glutathione (GSH) *S*-transferases in the presence of GSH to form non-mutagenic, stable *S*-(chrysen-5-yl)methylglutathione [31]. Similar evidence has been also obtained for the rapid scavenging effect of the rat liver cytosolic GSH *S*-transferases on the reactive sulphate esters of carcinogenic hydroxymethyl-BAs [10, 12, 32, 33]. The rat liver is not a target organ in carcinogenesis caused by the potent carcinogen, hydroxymethyl-BAs or methyl-BAs, as well as by 5-HCR or 5-MCR. On the contrary, in the mouse skin, a target organ in carcinogenesis induced by 5-HCR, the sulphate ester formed from 5-HCR was found to be hardly scavenged by GSH *S*-transferases because their activities in the skin were relatively low to those of the liver (data not shown).

It is of interest that in the examined extrahepatic

tissues, except guinea-pig adrenal, of the experimental animals used, the enzyme activities were higher in the female than in the male, as observed in their livers.

The authors have very recently obtained direct evidence for the participation of hydroxysteroid sulphotransferases in sulphate conjugation of 5-HCR and the carcinogenic hydroxymethyl-BAs, one of which was purified to homogeneity, had a subunit molecular weight of 30,500, catalysed the sulphation of the carcinogenic hydroxymethyl-arenes, and did neither catalyse the sulphation of *N*-OH-AAF nor 4-NP [34]. No appreciable 5-HCR-sulphating activity was found in the column-chromatographic fractions containing phenol sulphotransferases after separation of the hydroxysteroid sulphotransferases. These data will be published elsewhere after a further study.

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