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Sulfotransferase-Mediated Chlorination of 1-Hydroxymethylpyrene to a Mutagen Capable of Penetrating Indicator Cells

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Methylated polycyclic aromatic hydrocarbons are common in the human environment. Many of them are stronger carcinogens than their purely aromatic congeners. They may be metabolized to benzylic alcohols. We report here on biochemical and toxicological characteristics of 1-hydroxymethylpyrene (HMP), a typical representative of this class of compounds. Rat liver cytosol, fortified with 3'-phosphoadenosine-5'-phosphosulfate, converted HMP into its sulfate ester (HMPS). HMPS bound covalently to isolated DNA. In physiological buffer at 37°C, HMPS had a half-life of 2 min, the major decomposition product being HMP. Thus, cyclic activation is possible. When Cl\(^-\) or Br\(^-\) ions were present at physiological concentrations, an additional reaction product of HMPS, 1-chloromethylpyrene (CIMP), could be identified on the basis of its chromatographic properties and its mass spectrum, using the authentic standard for comparison. CIMP was shorter-lived in buffer than HMPS. CIMP reacted with DNA, the adduct pattern in the \(^{32}\)P-postlabeling analysis being similar, or identical, to that of HMPS. CIMP proved to be a very potent mutagen in *Salmonella typhimurium*, whereas HMPS, and HMP in the presence of a sulfate-conjugating system, showed strong mutagenicity only when Cl\(^-\) or Br\(^-\) ions were present in the exposure buffer. It is concluded that HMPS is capable of reacting with DNA, but is hampered in its distribution by membrane barriers. Strikingly, a CIMP intermediate is produced, which can act as a transport form to overcome membrane barriers. Among 10 investigated tissues, HMPS-activating sulfotransferases were found at appreciable levels only in the liver, and there the activity in parenchymal cells exceeded that in Kupffer cells by a factor of ≥ 200. Distribution processes and their restrictions may, therefore, be important factors determining the toxicology of benzylic alcohols and other compounds activated through conjugation with sulfate.

Introduction

Short-term tests have become a major tool in the detection of carcinogens and mutagens. In these systems, the active chemical species is frequently formed outside the indicator cell. If cell membranes act as barriers for a particular species, the result may be negative despite intrinsic reactivity toward important cell structures. Metabolic formation of such species within particular cells in the organism may produce tissue-specific toxicological effects. Difficulties in the penetration of membranes may be expected to exist, particularly with ionized chemical species such as sulfate conjugates.

Strong evidence has accumulated that sulfate conjugates play a major role in some carcinogenic effects of aromatic amines and alkenylbenzenes (1). Strikingly, however, the addition of a sulfate-conjugating system usually decreased the mutagenicity of aromatic amines and their metabolites in *Salmonella typhimurium*, whereas the covalent binding to isolated nucleic acids was enhanced (2–4). On the other hand, Watabe et al. (5–7) have observed DNA binding as well as bacterial mutagenicity for benzylic sulfate esters and alcohols, the latter requiring the presence of a sulfotransferase-containing metabolic system. To study structural and metabolic parameters governing this mutagenicity, we have synthesized a series of benzylic alcohols and sulfate esters. The exceedingly unusual characteristics that we have detected are described here for a representative benzylic alcohol, 1-hydroxymethylpyrene (HMP), and its sulfate ester, HMPS. This pair of compounds is of special interest, since the carbonium ion that results from heterolytic cleavage of the ester is stabilized by the same aromatic system as the
carbon ion formed from the bay-region diol-epoxide of benzo(a)pyrene, the most thoroughly investigated active metabolite of a polycyclic aromatic hydrocarbon.

The peculiarities of this mutagenicity include the occurrence of a cyclic activation process and the formation of a membrane-penetrating halogenated intermediate (the sulfate ester itself being practically incapable of entering the target cell). Furthermore, it has been found that the sulfotransferases which activate HMP occur only in few tissues, in particular in the liver, where they are further confined to certain cell types. Transport processes and their restrictions therefore appear to be essential factors in the toxicology of this class of compounds.

**Materials and Methods**

**Chemicals**

1-Pyrene carboxaldehyde was supplied by Aldrich Chemical Company (Steinheim, FRG). HMP was prepared from 1-pyrene carboxaldehyde by reduction with sodium borohydride in methanol. The product was purified by flash chromatography and finally recrystallized from n-hexane. HMPS was synthesized by sulfatation of HMP as described by Watabe et al. (5). Heating of HMP in the presence of thionyl chloride produced 1-chloromethylpyrene (CIMP). Treatment of the crude product with n-hexane furnished pure CIMP. 3'-phosphoadenosine-5'-phosphosulfate (PAPS) was purchased from Pharmacia LKB GmbH (Freiburg, FRG).

**Subcellular Preparations**

Unless otherwise indicated, adult male Sprague-Dawley rats, not treated with an enzyme inducer, were used. Tissues were homogenized in three volumes of ice-cold buffer B (150 mM KCl, 10 mM sodium phosphate buffer, pH 7.4), using an Elvehjem glass/tetlon homogenizer. The supernatant (S9) of a 9000g centrifuge (10 min) was separated into microsomal and cytosolic fractions by centrifugation at 100,000g for 1 hr. Cytosol was dialyzed for 3 hr against buffer B.

**Separation of Liver Cell Types**

Liver parenchymal cells were isolated using a two-step collagenase perfusion method (8). Nonparenchymal cell suspensions were prepared as described elsewhere (9). Endothelial and Kupffer cells were purified from nonparenchymal cell suspensions by centrifugal elutriation (10). Endothelial and Kupffer cell preparations contained < 0.5% parenchymal cells, whereas the parenchymal cell preparations contained < 5% nonparenchymal cells.

**Mutagenicity Assay**

The his\(^{-}\) *Salmonella typhimurium* strain TA98 was grown and resuspended in medium A (1.6 g Bacto nutrient broth and 5 g NaCl/L) as described previously (11). In the direct test, buffer B (500 µL), bacterial resuspension (100 µL), and the test compound (added in 10 µL dimethylsulfoxide) were mixed in a glass tube and incubated for 20 min at 37°C. Then, 2 mL of 45°C warm soft agar (0.55% agar, 0.55% NaCl, 50 µM histidine, 50 µM biotin, 25 mM sodium phosphate buffer, pH 7.4) was added, and the mixture was poured onto a Petri dish containing 24 mL minimal agar (1.5% agar in Vogel-Bonner E medium with 2% glucose). After incubation for 3 days in the dark, colonies (his\(^{+}\) revertants) were counted.

In the experiments in which the stability of mutagens in the incubation buffer was studied, the assay was modified in that the bacteria were added to the buffer 0 to 20 min after the addition of the test compounds (12).

In the enzyme-mediated test, the procedure was as above, but buffer B was replaced in part by cytosol (167 µL in the standard assay; variable amounts in the comparison of cytosols from different tissues) and cofactor solution (10 mM ATP, 10 mM Na\(_2\)SO\(_4\), 6 mM MgCl\(_2\) in the total incubation volume, 610 µL). In some cases, ATP was replaced by PAPS (5–100 µM).

The compounds (Table 1) or the enzyme preparations (Table 2) were tested at 5 to 15 concentrations, usually with 3 incubations for each treatment group. Revertants per nanomole test compound (specific mutagenicities) and revertants per milligram protein of the enzyme preparation were calculated from the increasing part of the concentration-response curve as described previously (11).

**32P-Postlabeling Analysis**

The test compound (at concentrations of 0.1, 1, and 10 µg/mL) was incubated at 37°C in buffer B or 10 mM sodium phosphate buffer (pH 7.4), as described for the mutagenicity assay, except that the bacteria were replaced by salmon sperm DNA (10 µg/mL). The DNA was then isolated, digested and \(^{32}\)P-postlabeled, and the nucleotides were chromatographically separated as described previously (12).

**Identification of Products Formed from HMPS in Buffer**

HMPS (1.2 mM) was incubated in buffer B or H\(_2\)O for 20 min (equivalent to 10 half-lives). The incubate (1 mL) was then extracted twice with an equal volume of ethyl acetate. The extract was dried over Na\(_2\)SO\(_4\) and the solvent evaporated. The residue was chromatographed on silica-gel thin layer plates using a mixture of chloroform/methanol (19:1; v/v); HMPS, HMP, and 1-pyrene carboxaldehyde were used as reference compounds.

In a second experiment, a continuous extraction of the incubate with n-hexane was performed for 20 min (10 half-lives) starting immediately after the addition of the test compound. After drying over Na\(_2\)SO\(_4\) the extract was concentrated, and an aliquot was analyzed by gas chromatography-mass spectrometry (GC-MS). GC-MS was performed on a Finnigan MAT model Incos60 quadrupole mass spectrometer (EI mode; 70 eV); gas chromatograph (Varian 3400) interfaced to an INCOS data system. The
Table 1. Mutagenicity in Salmonella typhimurium TA98 of 1-hydroxymethylpyrene and derivatives under various experimental conditions.

| Compound                  | Exposure medium*                                      | Specific mutagenicity, revertants/nmole* |
|---------------------------|-------------------------------------------------------|----------------------------------------|
| 1-Hydroxymethylpyrene     | KCl, 125 mM; Na phosphate buffer, pH 7.4, 8 mM*       | < 0.01                                 |
|                           | Postmitochondrial fraction, NADPH^                    | 9                                      |
|                           | Cytosol, PAPS                                          | 970°                                   |
|                           | Cytosol, ATP                                           | 770, 900, 1160°                        |
|                           | Cytosol, no cofactor                                   | < 0.1                                  |
|                           | PAPS, no cytosol                                       | < 0.1                                  |
|                           | ATP, no cytosol                                        | < 0.1                                  |
|                           | Cytosol, ATP, no chloride                              | 30°                                   |
| 1-Hydroxymethylpyrene sulfate | KCl, 125 mM; Na phosphate buffer, pH 7.4, 8 mM*       | 250, 530, 450                          |
|                           | H2O                                                   | 80°                                    |
|                           | KCl 250 mM                                            | 760°; 360°                            |
|                           | KCl 125 mM                                            | 760°; 540°                            |
|                           | KCl 62 mM                                             | 610°; 370°                            |
|                           | KCl 31 mM                                             | 290°; 190°                            |
|                           | NaCl 125 mM                                           | 470°; 180°                            |
|                           | MgCl₂ 62 mM                                           | 150°; 90°                             |
|                           | K₂SO₄ 62 mM                                           | 15°                                   |
|                           | K acetate 125 mM                                       | 80°                                   |
|                           | K phosphate buffer, pH 7.4, 62 mM                      | 13°                                   |
|                           | Na₂SO₄ 62 mM                                          | 56°                                   |
|                           | Na phosphate buffer, pH 7.4, 80 mM                    | 35°                                   |
|                           | Glucose 250 mM                                        | 56°                                   |
| 1-Pyrene carboxaldehyde   | KCl, 125 mM; Na phosphate buffer, pH 7.4, 8 mM*       | 46,000; 31,000                       |
| 1-Chloromethylpyrene      | KCl, 125 mM; Na phosphate buffer, pH 7.4, 8 mM*       | 9.1°                                  |

*Unless indicated otherwise, bacteria were added in resuspension medium, adding 14 mM NaCl (final concentration) and Bacto nutrient broth (0.25 mg/mL) to the exposure medium.

Each value represents a separate experiment. For negative results, detection limits are given. Differences in the detection limits may be because the maximal concentrations used were different.

Unless indicated otherwise, bacteria were added in resuspension medium, adding 14 mM NaCl (final concentration) and Bacto nutrient broth (0.25 mg/mL) to the exposure medium.

Classical S9 mix, prepared from liver of Aroclor 1254-treated Sprague-Dawley rat, was used. Exactly the same protocol as in a previous study (12) was used.

The bacteria were resuspended in distilled water.

A Wistar rat was used.

Isotonic sucrose solution was used as the homogenization buffer. MgCl₂ and KCl were replaced by MgSO₄ and potassium phosphate (pH 7.4) buffer, respectively.

sample in n-hexane was injected in the splitless mode, with the injector temperature at 210°C, onto a 15-m DB-1 capillary column with 0.25 mm internal diameter (5% methyl silicone as liquid phase), with helium as the carrier gas (flow rate: 3 mL/min) and eluted with a temperature gradient (20°C/min) from 70°C to 260°C.

Results

Mutagenicity in Salmonella typhimurium TA98

HMP and its oxidation product, 1-pyrene carboxaldehyde, were not mutagenic in the direct test. However, when NADPH-fortified postmitochondrial fraction from the liver of Aroclor 1254-treated rats (the classical activating system of the Ames test) or PAPS-fortified rat liver cytosol was added, the number of mutants per plate was increased about 100-fold above background (from 30 to about 3000 colonies) at optimal dose of HMP. The initial part of the dose-response curves was slightly hyperlinear, whereas at high doses mutagenicity was overshadowed by bacteriotoxicity. For the induction of equal effects, about 100 times higher concentrations of HMP were required with postmitochondrial fraction and NADPH than with cytosol and PAPS, resulting in corresponding differences in the specific mutagenicity (Table 1). Due to the high cost of PAPS, it was usually replaced by ATP, serving as substrate for the in situ generation of PAPS in the system used. Similar mutagenicities were observed with these cofactors (except when the cytosol was used at limiting concentrations). Cytosol in the absence of cofactor and PAPS and ATP in the absence of cytosol were fully devoid of any activating effect on HMP. These observations strongly suggested the involvement of sulfotransferases.

Authentic HMPS showed direct mutagenic activity, the maximal effect and the shape of the concentration-response curve being similar to that of bioactivated HMP. It was somewhat surprising that the specific mutagenicity of HMPS only amounted to about one-third of that of metabolically activated HMP (Table 1) and was further reduced in the presence of cofactor-free cytosol. On the other hand, fortification of the cytosol with ATP resulted in a 3-fold enhancement of the mutagenicity of HMPS (data not shown). This suggests that not only HMP, but also HMPS or a metabolite(s), are subjected to sulfotransferase-mediated activation.
Table 2. Activation of 1-hydroxymethylpyrene by cytosol from various tissues and cells from Sprague-Dawley rats.*

| Tissue, cell    | Cofactor | Revertants/mg tissue equivalenta |
|----------------|----------|---------------------------------|
| Preparations from males |          |                                 |
| Liver Parenchymal cells | ATP      | 1240; 1370; 1770; 2100          |
| Kupffer cells        | ATP      | 1080*                           |
| Endothelial cells    | ATP      | 5*                              |
| Lung                 | ATP      | < 0.3                           |
| Small intestine      | ATP      | < 0.3                           |
| Spleen               | ATP      | < 0.3                           |
| Adrenal gland        | ATP      | < 1                             |
| Heart                | ATP      | < 0.3                           |
| Brain                | ATP      | < 0.3                           |
| Testis               | ATP      | 4; 10                           |
| Preparations from females |      |                                  |
| Liver                | ATP      | 2480                            |
| Liver                | PAPS     | 10,000                          |
| Kidney               | PAPS     | 115                             |
| Lung                 | PAPS     | < 0.3                           |
| Small intestine      | PAPS     | < 0.3                           |
| Adrenal glandb       | PAPS     | 2.7                             |
| Heart                | PAPS     | 0.7                             |
| Mammaryc             | PAPS     | 1.5                             |

a A constant dose of test compound (10 μg) was incubated with various concentrations of dialyzed cytosol in the presence of 10 mM ATP (which should give rise to the formation of PAPS under the experimental conditions) or of 5 μM PAPS. After subtraction of the background value, the number of mutants was divided by the amount of cytosol added. Multiple values represent experiments carried out on separate occasions. For negative results the detection limit is shown. 
b Results for the different liver cell types are expressed per 100 μg cytosolic protein. 
c Mitochondrial fraction instead of purified cytosol was used.

It was conspicuous that the mutagenicity of HMPS was strongly dependent on the composition of the exposure medium, especially on the presence and the nature of anions (Table 1). In the absence of chloride, bromide, and acetate anions, the mutagenicity was reduced to 2% of that under standard conditions in which chloride was present at a concentration of 139 mM. Bromide and, with lower efficiency, acetate, could substitute for chloride, whereas sulfate and phosphate were without effect. The influence of cations was much less than that of anions. In all exposure media used, the mutagenicity of the positive control, benzo(a)pyrene 4,5-oxide, varied only within a factor of 2.5 (data not shown), which excludes nonspecific mechanisms, such as bacteriotoxicity of medium constituents, for the differences observed with HMPS. Replacement of the chloride ions not only reduced the mutagenicity of HMPS, but also that of bioactivated HMP (Table 1). CIMP turned out to be an extremely potent, direct-acting mutagen in Salmonella typhimurium (Table 1). Its potency exceeded those of all investigated diol-epoxides and other metabolites of polycyclic aromatic hydrocarbons (14). Under our standard conditions (buffer B), CIMP was about 100 times more active than HMPS, the difference being even larger in chloride-free medium.

Stability in the Exposure Medium

The stabilities were determined from the decline in mutagenicity after preincubation of the test compound in the absence of the bacteria for varying times. In buffer B at 37°C, the decline in mutagenicity of HMPS followed first-order kinetics, with a half-life of 2 min. The same kinetics were observed in distilled water, within the limits of accuracy resulting from the low mutagenicity under these conditions. CIMP was less stable than HMPS. Preincubation for 20 sec in buffer B resulted in a more than 50% decrease in mutagenicity.

Identification of the Products Formed from HMPS in Buffer

HMPS was incubated for 10 half-lives in buffer B. After this time, virtually all material with pyrenelike chromophores was extractable. Thin-layer chromatography of the extract showed two spots with the Rf values of HMP and 1-pyrene carboxaldehyde. When HMP was subjected to the same incubation and extraction procedure, the aldehyde was formed as well, indicating a significant autoxidation of HMP.

In a second experiment, extraction was started simultaneously with the addition of HMPS. In this case, the thin-layer chromatogram showed a third spot with the Rf value of CIMP. The extract was then analyzed by GC-MS. A peak was found which had the retention time and the mass spectrum of CIMP (Fig. 1). The molecular ions were observed at 250 and 252 m/z with the characteristic 3:1 ratio, corresponding to the natural isotope distribution of chlorine. The base peak, at 215 m/z, corresponds to the benzylic carbonium ion.

32P-Postlabeling Analysis of DNA Incubated with HMPS and CIMP

Both HMPS and CIMP formed adducts when they were incubated with salmon sperm DNA in chloride-free buffer. The two adduct patterns, resolved by four-dimensional thin-layer chromatography, consisted of four major spots and were indistinguishable. The adduct level was, however, higher for CIMP. Addition of KCl (150 mM) to the reaction mixture did not affect adduct level and pattern of either compound.

Tissue and Cell Distribution of HMP-Activating Sulfotransferases

Among seven investigated extrahepatic tissues of male rats, only ATP-fortified cytosol from testis activated HMP to a mutagen (Table 2). The amount of testis protein required for induction of an equal effect was more than 100 times higher than for liver. Among different liver cell types, the parenchymal cells showed the highest levels of activity. Substantial levels were present in endothelial cells as well, whereas those in Kupffer cells were very
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and probably involved microsomal oxidative metabolism. The second pathway, yielding much higher mutagenicity, required the presence of cytosol and PAPS (or its precursor, ATP). Authentic HMPS proved to be chemically reactive. It reacted with water and other nucleophiles including DNA and halogen anions. Reaction of HMPS with water led to the formation of HMP, the metabolic precursor of HMPS. Cyclic activation therefore appears possible. This idea was supported by the observations that HMP in the presence of cytosol and ATP was more mutagenic than HMPS in the absence of a hepatic metabolizing system and that the mutagenicity of HMPS in the presence of cytosol was increased on addition of ATP. The mutagenicity results indicated the occurrence of about three rounds of activation under our experimental conditions. Cyclic metabolism not only enhances the risk of reaction with important tissue molecules, it also should lead to waste of energy through conversion of active sulfate (PAPS) to inorganic sulfate.

HMPS reacted with DNA and appeared to form the same adducts as did the strong mutagen, CIMP. Nevertheless, HMPS was only very weakly mutagenic, unless chloride anions or similar nucleophiles were added to the medium. These findings suggest that extracellularly generated HMPS itself has little or no access to the bacterial DNA. It is probable that the cell membrane acts as a barrier for the ionized sulfate ester and the potentially formed benzylic carbonium ion.

Formation of CIMP was observed on incubation of HMPS in chloride-containing buffer. CIMP was highly unstable in water, reacted with DNA, and proved to be an extraordinarily potent mutagen in Salmonella typhimurium. The strongly potentiating influence of chloride anions on the mutagenicity of HMPS indicated that CIMP probably acted as the transport form of the mutagen. Calculation of the specific mutagenicities of HMPS and CIMP in the presence of chloride (buffer B) and of HMPS in chloride-free buffer indicates that about 1% of HMPS was converted to CIMP. Although this amount may be considered small, it was of great toxicological significance in the assay. Since the chloride concentration, pH, and temperature used were in the physiological range, this reaction may also occur in vivo. At least the cytosol-mediated activation of HMP was dependent on the presence of chloride anions, demonstrating that liver enzymes do not prevent the reaction.

The finding that cytosol-mediated activation of HMP is dependent on the presence of chloride anions indicates the occurrence of a metabolic halogenation of HMP. To our knowledge this is the first observed case of metabolic chlorination of a xenobiotic in a mammalian enzyme system.

The strong effect of halogen anions on the mutagenicity of HMPS was unexpected and observed accidentally. It demonstrates that apparently unimportant details of the protocol may have a profound influence on the test results. Unfortunately, many authors reporting short-term tests do not describe their method in detail, but only refer to

Figure 1. Mass spectra of a metabolite extracted from an incubation of 1-hydroxymethylpyrene sulfate in buffer B (150 mM KCl, 20 mM sodium phosphate buffer, pH 7.4) (upper panel) and authentic 1-chloromethylpyrene (lower panel).

Discussion

Biotransformation of HMP to Mutagenic Species

HMP was activated to mutagens by at least two different metabolic routes. The first pathway occurred in the presence of the postmitochondrial fraction and NADPH
review papers, not allowing reconstruction of the exact protocol. Thus, their results may not be reproducible by others and sometimes not even interpretable.

Toxicological Implications of Hampered Transport of Active Species

A number of compounds are known that are activated through conjugation with sulfate to their ultimate carcinogenic and genotoxic metabolites (1). To our knowledge, benzylic alcohols are the only group for which a positive result has been obtained in a short-term test using an exogenous sulfate-conjugating system for the activation (5-7). In the present study, it was demonstrated that this effect involves a special mechanism, namely, substitution of the polar sulfate group by a nonpolar halogen group. When this substitution was not possible, HMPS reacted with free DNA, but was only a poor mutagen, apparently because of problems in entering the indicator cell. It is reasonable to assume that not all reactive sulfate conjugates may be converted into a transport form. Their toxicological effects therefore may be restricted to the cell where they are formed, and possibly even some of its compartments. And since sulfotransferases, as shown here for the HMP-activating enzymes, are present at high levels in only few cell types in the organism, high target tissue selectivity may result. This implies that effects may be absent in common target tissues used in in vivo short-term tests, such as bone marrow and skin. The likelihood is also high that false negative results occur in tests with bacteria and cells in culture using exogenous activating systems. Taking into account the difficulties in detection, very little is known about how many chemicals are activated to reactive sulfates and, in general, to active metabolites incapable of penetrating membranes.

Perspectives

The barrier problem requires a special approach for the detection of sulfotransferase-mediated toxicological effects. The first possibility is offered by cell-free systems, using, for example, binding to free DNA as the end point. The alternative is sulfotransferase-expressing indicator cells, in particular freshly isolated hepatocytes. The involvement of sulfotransferases may be examined by comparing the effect in control and cofactor-depleted cells. The most relevant end points with regard to genotoxicity can be studied only in proliferating cells and therefore hepatocytes are not suitable. Recently, cytochromes P-450 were introduced and permanently expressed in V79 cells through gene transfer (14,15). Various compounds have proved to be mutagenic in these cells but inactive in parent V79 cells. The same approach is being used for sulfotransferases. A 1.0 kbp cDNA fragment of a HMP-activating sulfotransferase has been cloned and is being used in the search for a full-length cDNA clone. Pairs of sulfotransferase-expressing and nonexpressing cell lines should provide further insight into the role of this enzyme in metabolic toxification and into barriers affecting the toxic effects.

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REFERENCES

1. Miller, E. C., Miller, J. A., Boberg, E. W., Delelos, K. B., Lai, C.-C., Fennell, T. R., Wiseman, R. W., and Liem, A. Sulfuric acid esters as ultimate electrophilic and carcinogenic metabolites of some alkenylenzenes and aromatic amines in mouse liver. Carcinog. Compr. Surv. 10: 93-107 (1985).
2. Mulder, G. J., Hinson, J. A., Nelson, W. L., and Thorgeirsson, S. S. Role of sulfotransferase from rat liver in the mutagenicity of N-hydroxy-2-acetylaminofluorene in Salmonella typhimurium. Biochem. Pharmacol. 26: 1356-1358 (1977).
3. Andrews, L. S., Hinson, J. A., and Gillette, J. R. Studies on the mutagenicity of N-hydroxy-2-acetylaminofluorene in the Ames-Salmonella mutagenesis test system. Biochem. Pharmacol. 27: 2399-2408 (1978).
4. Glatt, H. R., Oesch, F., and Neumann, H.-G. Factors responsible for the metabolic formation and inactivation of bacterial mutagens from trans-4-acetylaminostilbene. Mutat. Res. 73: 297-309 (1980).
5. Watabe, T., Ishizuka, T., Isobe, M., and Ozawa, N. A 7-hydroxymethyl sulfate ester as an active mutagen of 7,12-dimethylbenz(a)anthracene. Science 215: 405-406 (1982).
6. Watabe, T., Ishizuka, T., Hakamata, Y., Aizawa, T., and Isobe, M. Covalent binding of the proximal nucleophile, 7-hydroxy-12-methylbenz(a)anthracene (7-HMBA) to rat liver cytosolic protein via 7-HMBA sulphate. Biochem. Pharmacol. 32: 2120-2122 (1983).
7. Okuda, H., Nojima, H., Miwa, K., Watanabe, N., and Watabe, T. Selective covalent binding of the active sulfate ester of the carcinogen 5-(hydroxymethyl)chrysene to the adenine residue of calf thymus DNA. Chem. Res. Toxicol. 2: 15-22 (1989).
8. Glatt, H. R., Billings, R., Platt, K. L., and Oesch, F. Improvement of the correlation of bacterial mutagenicity with carcinogenicity of benzo(a)pyrene and four of its major metabolites by activation with intact liver instead of cell homogenate. Cancer Res. 41: 270-277 (1981).
9. Praeving-van Dalen, D. P., and Knook, D. L. Quantitative determination of in vivo endocytosis by rat liver Kupffer and endothelial cells facilitated by an improved cell isolation method. FEBS Lett. 141: 229-232 (1982).
10. Steinberg, P., Lafranconi, W. M., Wolf, C. R., Waxman, D. J., Oesch, F., and Friedberg, T. Xenobiotic metabolizing enzymes are not restricted to parenchymal cells in rat liver. Mol. Pharmacol. 32: 463-470 (1987).
11. Glatt, H. R. Mutagenicity spectra in Salmonella typhimurium strains of glutathione, L-cysteine and active oxygen species. Mutagenesis 4: 221-227 (1989).
12. Glatt, H. R., Harvey, R. G., Phillips, D. H., Hewer, A., and Grover, P. L. Influence of the alky substituent on mutagenicity and covalent DNA-binding of bay-region diol-epoxides of 7-methyl- and 7-ethylbenzo(a)anthracene in Salmonella and V79 Chinese hamster cells. Cancer Res. 49: 1778-1782 (1989).
13. Glatt, H. R., and Oesch, F. Structural and metabolic parameters governing the mutagenic potency of polycyclic hydrocarbons. In: Chemical Mutagens: Principles and Methods for Their Detection, Vol. 10 (F. J. de Serres, Ed.), Plenum Press, New York, 1986, pp. 73-127.
14. Doehmer, J., Dogra, S., Friedberg, T., Monier, S., Adesnik, M., Glatt, H. R., and Oesch, F. Stable expression of rat cytochrome P4501B1 cDNA in Chinese hamster cells (V79) and mutagenicity testing of polycyclic aromatic hydrocarbons. Proc. Natl. Acad. Sci. USA 86: 57-61 (1989).
15. Glatt, H. R., Gemperlein, I., Turchi, G., Heinritz, H., Doehmer, J., and Oesch, F. Search for cell culture systems with diverse xenobiotic-metabolizing activities and their use in toxicological studies. Mol. Toxicol. 1: 313-334 (1987).