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# SPECIFIC ALTERATIONS OF RAT RENAL MICROSOMAL PROTEINS INDUCED BY CEPHALORIDINE

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**Abstract**—In order to elucidate the molecular mechanisms of cephaloridine (CPH) nephrotoxicity, the effect of cephaloridine treatment on the protein composition of different subcellular fractions from rat kidney cortex was investigated. After intravenous treatment of male Wistar rats with 250–1200 mg/kg/d CPH for 1–3 days, kidneys were removed and the homogenate from renal cortex was separated into lysosomal, cytosolic and microsomal fractions. The polypeptide composition of the different subfractions was analyzed by one-dimensional SDS-gel electrophoresis and quantified by densitometry. Significant differences in the polypeptide composition between treated and non-treated animals were seen in the microsomal fraction. CPH-treatment induced a polypeptide with an apparent molecular weight of 44,000 and decreased the content of cytochrome P-450 isoenzymes in the microsomal fraction. Solubilization experiments showed that the CPH-induced microsomal polypeptide of molecular weight 44,000 is a peripheral membrane protein rather than an integral membrane protein. The induction of this protein by CPH was dose- and time-dependent. Preliminary experiments using the kidney slice technique indicate that the induction of this polypeptide correlates with the nephrotoxicity measured as decrease in renal cortical accumulation of organic ions. Thus, the results of the present study indicate that treatment of rats with CPH resulted in the induction of a microsomal polypeptide of molecular weight 44,000 which could be a sensitive parameter of cephaloridin nephrotoxicity.

Cephaloridine (CPH) is a β-lactam antibiotic which may cause acute proximal tubular necrosis in animals and humans [1, 2]. The exact biochemical mechanisms of cephaloridine-induced nephrotoxicity are not known. In a recent study [3] it has been shown that CPH leads to generation of reactive oxygen species which may react with membrane lipids and induce lipid peroxidation [4–6]. The peroxidation of lipids in biochemical systems is a destructive process which causes cellular damage [7]. In contrast to the well studied effects of CPH on membrane lipids, little or no attention was payed to the possible effects of CPH on the protein composition of various cellular constituents. Therefore, the purpose of the present study was to investigate whether treatment of rats with CPH alters the protein composition of various subcellular fractions from kidney cortex cells.

## MATERIALS AND METHODS

*Materials.* CPH and molecular weight standards used for electrophoresis were obtained from Sigma (München, F.R.G.). Acrylamide, *N,N'*-bisacrylamide, Serva Blue R 250 and nonionic detergents were obtained from Serva (Heidelberg, F.R.G.). All other chemicals were purchased from the usual commercial sources with the highest purity available.

*Treatment of animals.* Male Wistar rats (Hoechst AG, Frankfurt/Main, F.R.G.) weighing 250–350 g were used. Rats were maintained on a standard diet (Altromin®) with free access to water. Rats were treated intravenously with 250, 500 or 1200 mg/kg/d CPH for 3 days or with 1200 mg/kg/d for 1, 2 or 3 days. Control rats were given the corresponding volume of the vehicle (0.9 g% sodium chloride solution). In another series of experiments, rats were given phenobarbital (80 mg/kg/d) intraperitoneally for 3 days. Each treatment group consisted of at least five rats. Animals were killed by cervical dislocation, kidneys were removed, the renal cortex was homogenized and the homogenate was centrifuged at 500 *g* for 5 min at 4°. The supernatant was centrifuged at 15,000 *g* for 15 min at 4°. The resulting pellet containing lysosomes, mitochondria and peroxisomes is referred to herein as the lysosomal fraction. Cytosolic and microsomal fractions were prepared from the kidney cortex as described previously [8] using a phosphate buffer [9]. These subcellular fractions were used the same day or they were stored at −80° for subsequent analysis of the polypeptide composition as described below. Protein concentration was determined according to Bradford [10] using the Bio-Rad test kit (Bio-Rad München, F.R.G.).

*Solubilization of the subcellular fractions.* For solubilization of rat renal cortical microsomes, 200–300 μg of microsomes were treated with 300 μl of a 1% solution of Triton X-100 in 10 mM Tris/Hepes buffer, pH 7.4. After 30 min of incubation at 4°, solubilized material was separated from nonsolubilized membranes by centrifugation at 48,000 *g* for 30 min. Supernatant and pellet were adjusted to 400 μl with distilled water and protein was precipitated with chloroform/methanol according to Wessel and Flügge [11]. Phase separation experiments with Triton X-114 were performed by a modification of the Bordier method [12] according to Tirrupathi *et al.* [13]. One milligram of microsomal membranes were solubilized with 200 μl of a 1% solution of Triton X-114 in 20 mM Tris/HCl buffer,

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pH 7.4 containing 1 mM PMSF, 140 mM NaCl and incubated at 4° for 10 min. After centrifugation at 48,000 *g* for 60 min, the clear supernatant was overlaid to 100 μl of 0.06% Triton X-114, 6% sucrose in 20 mM Tris/HCl buffer, pH 7.4 in a 1.5 ml reaction tube. After 5 min incubation at 32°, the tube was centrifuged for 5 min at 3000 *g* at 22°. The upper phase (detergent poor phase) was separated from the detergent rich phase (lower phase). Both phases were adjusted to 300 μl with distilled water and the protein was precipitated [11]. Separation of integral from peripheral membrane proteins by alkaline treatment was performed according to Fujiki *et al.* [14]. Five hundred micrograms of rat renal cortical microsomes were suspended in 200 μl of 100 mM sodium carbonate and kept on ice for 30 min. After centrifugation at 48,000 *g* for 30 min, the supernatant containing peripheral membrane proteins was separated from nonsolubilized membranes. The membrane pellet and the supernatant were adjusted to 400 μl with distilled water and the protein was precipitated as described above.

*SDS-gel electrophoresis.* The protein composition of the renal cortical microsomes and of the solubilized subfractions, prepared as described above was analyzed by SDS gel electrophoresis [15]. The dried protein precipitates were dissolved in 40 μl of 62.5 mM Tris/HCl buffer, pH 6.8 containing 5% 2-mercaptoethanol, 2% SDS, 10% glycerol and 0.001% bromophenol blue. After heating of the samples to 90° for 5 min and centrifugation at 15,000 *g* for 10 min, the clear supernatants were submitted to SDS-gel electrophoresis on 0.7 × 150 × 200 mm slab gels using a Pharmacia L2/4B apparatus (Pharmacia, Freiburg, F.R.G.) [16]. Total acrylamide concentration was 7.5–12% with a ratio 2.8% *N,N'*-bis acrylamide and 97.2% acrylamide. Gels were run at a constant voltage of 60 V and after electrophoresis fixed in 12.5% trichloroacetic acid. After staining with 0.8% Serva Blue R 250 in 30% methanol/9% acetic acid solution and destaining in 30% methanol/9% acetic acid, the gels were stored in 5% acetic acid. Densitometric scanning of the gels was performed at 595 nm with a DESAGA CD 50 densitometer (DESAGA, Heidelberg, F.R.G.).

## RESULTS

### *Subcellular localization of the CPH-induced alterations in polypeptide composition*

After intravenous treatment of rats with CPH (1200 mg/kg/d) for 3 days, no relevant changes in the polypeptide composition of the lysosomal and cytosolic fractions could be observed by SDS gel electrophoresis. However, the polypeptide pattern of the renal cortical microsomes differed markedly between CPH-treated and control animals.

### *Induction of a renal microsomal polypeptide of molecular weight* 44,000 *by CPH*

The densitograms in Fig. 1 shows that the intensity of a polypeptide with apparent molecular weight 44,000 was markedly increased in renal microsomes from CPH-treated animals as compared to controls. Additionally, a clear decrease of stained polypeptides in the molecular weight region of 50–53,000

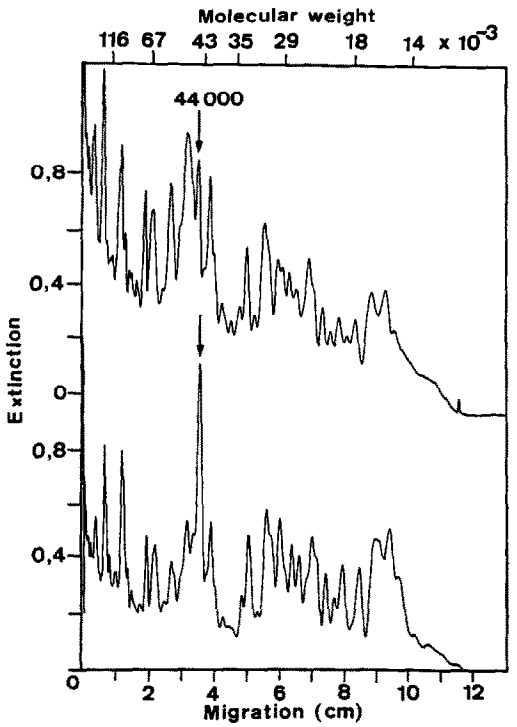


Fig. 1. Densitogram of the rat renal microsomes from control and CPH-treated rats after SDS-gel electrophoresis. Microsomes from rat kidney cortex were submitted to SDS-gel electrophoresis on 10.5% acrylamide gels. The upper curve shows the distribution of Serva Blue R-250 stained microsomal polypeptides from control rats, whereas the lower curve that of CPH-treated rats (1200 mg/kg/d for 3 days).

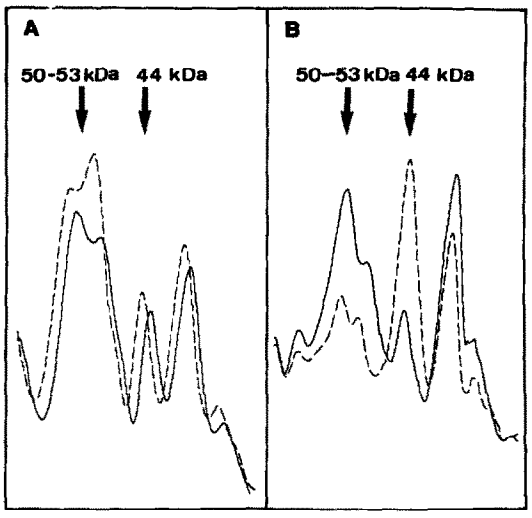


Fig. 2. Densitograms of renal microsomes from rats treated either with phenobarbital or with CPH. Rats were treated for 3 days with 80 mg/kg/d phenobarbital, intraperitoneally (A) or with 1200 mg/kg/d CPH (B), intravenously. Subsequently, the renal cortical microsomes were isolated and submitted to SDS-gel electrophoresis on 9% gels. The dotted lines show the distribution of Serva Blue stained polypeptides in microsomes from CPH-treated animals, whereas the solid lines show the distribution of stained polypeptides from control animals.

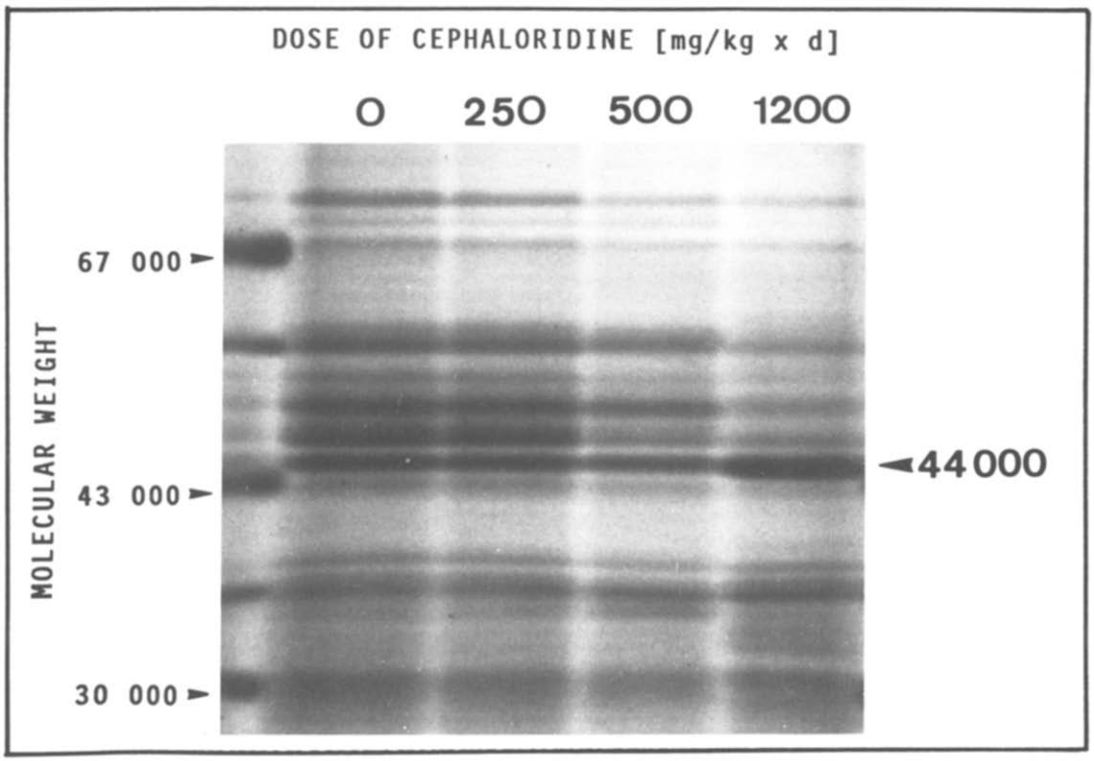


Fig. 3. Dose dependency of CPH-induced alterations in polypeptide composition of the renal cortical microsomes. Rats were treated intravenously for 3 days with 250, 500 or 1200 mg/kg/d CPH. Subsequently, the renal microsomal fractions were submitted to SDS-gel electrophoresis.

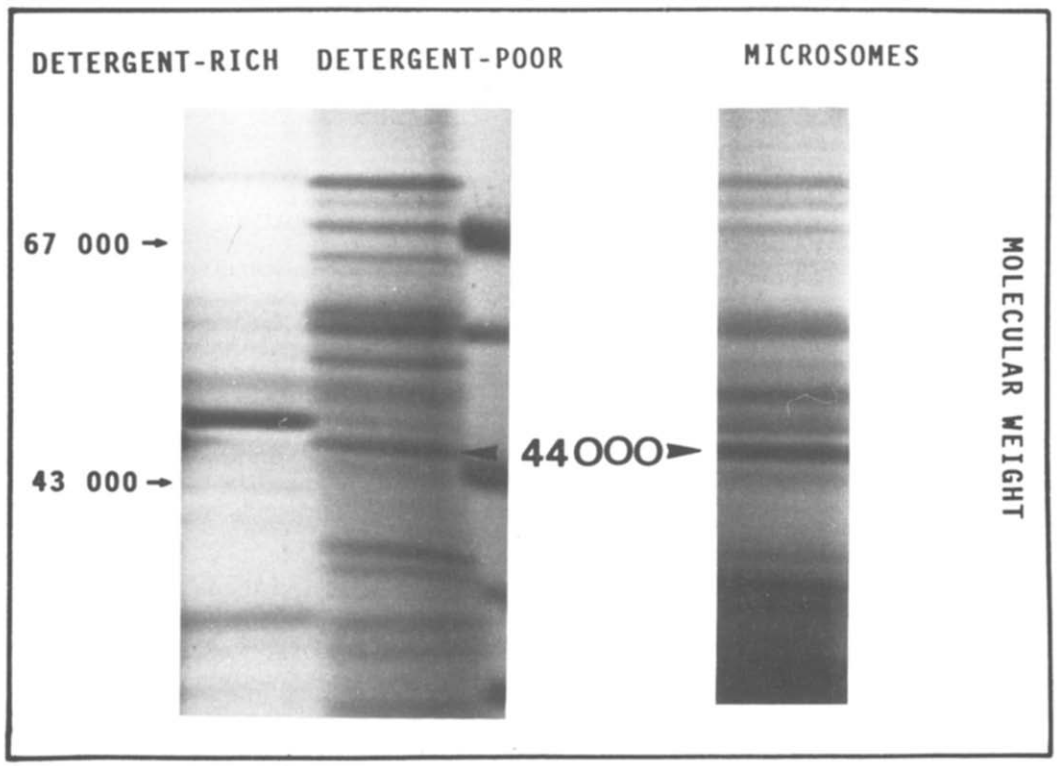


Fig. 4. Phase separation of renal microsomal proteins with Triton X-114. Renal cortical microsomes from rats treated intravenously with CPH (1200 mg/kg/d for 3 days), were solubilized with Triton X-114. After phase separation the detergent poor and the detergent rich fractions were submitted to SDS gel-electrophoresis.

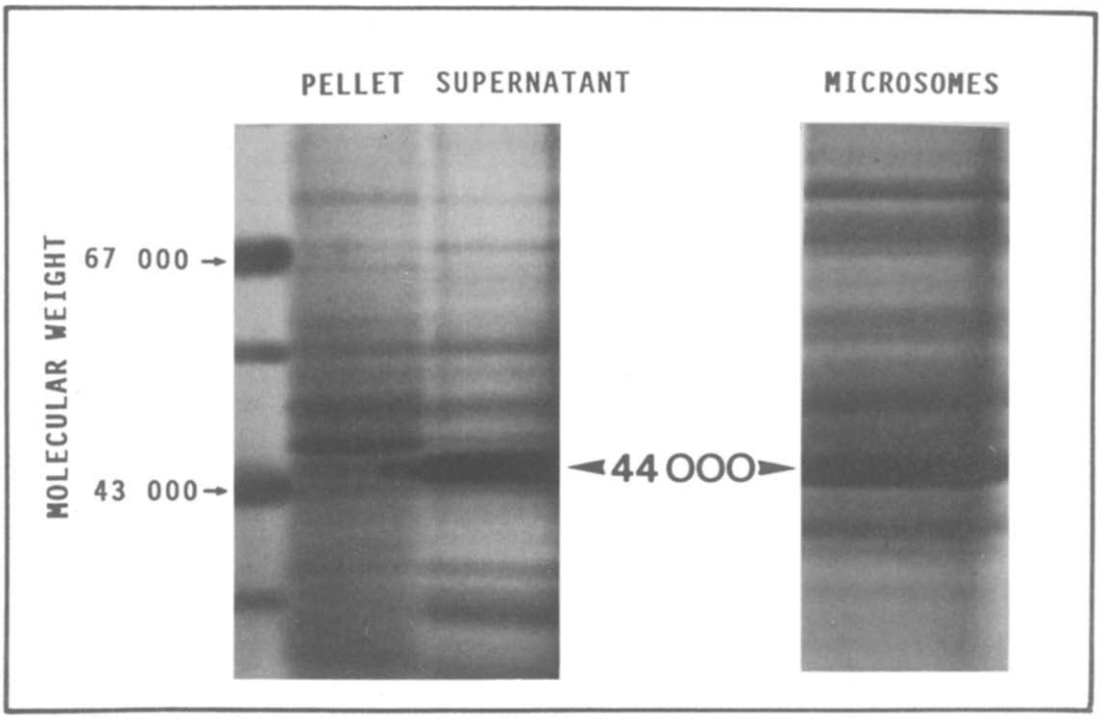


Fig. 5. Alkaline extraction of the rat renal cortical microsomes. Renal cortical microsomes prepared from rats treated intravenously for 3 days with 1200 mg/kg/d CPH were incubated with 100 mM sodium carbonate. Solubilized proteins were separated from nonsolubilized material and both fractions were submitted to SDS-gel electrophoresis.

was observed. Treatment of control rats with phenobarbital (80 mg/kg/d), a known inducer of cytochrome P-450 [17], led to an increase in the intensity of polypeptides of molecular weights 50–53,000, but no relevant increase in the intensity the 44,000 polypeptide occurred (Fig. 2a). However, in a parallel experiment, treatment of rats with CPH (1200 mg/kg/d) for 3 days increased the intensity of the 44,000 molecular weight polypeptide and simultaneously decreased the staining of polypeptides in the 50–53,000 molecular weight region (Fig. 2b). The amount of the 44,000 molecular weight polypeptide increased in the renal cortical microsomes in a time- (data not shown) and dose-dependent fashion (Fig. 3). *In vitro* incubation of renal cortical microsomes from control rats with CPH (1.25–10 mg/ml) for 1 hr had no effect on the polypeptide pattern when compared to controls (data not shown).

*Further characterization of the CPH-induced polypeptide*

For further characterization, the 44,000 molecular weight polypeptide was solubilized from the renal cortical microsomes with non-ionic detergents and sodium carbonate. The 44,000 molecular weight polypeptide could be solubilized by treatment of renal microsomes with 1% Triton X-100 (data not shown). After phase separation with Triton X-114, the 44,000 molecular weight polypeptide was found in the detergent poor phase (Fig. 4). Treatment of microsomes with 100 mM sodium carbonate completely detached the 44,000 molecular weight polypeptide from the microsomal membranes (Fig. 5). These experiments indicate that the 44,000 molecular weight polypeptide induced by CPH in the renal cortical microsomes is a peripheral membrane protein.

## DISCUSSION

The nature and the extent of the biological responsiveness of a subcellular organelle to xenobiotics depend upon the presence of specific interactions of the respective xenobiotic with components of the target organelle. The extent of cephaloridine (CPH) nephrotoxicity appears to be directly related to the cortical concentration of this cephalosporin in the kidney cortex [18, 19]. Various studies showed that inhibitors of the organic anion transport reduce both renal cortical accumulation and nephrotoxicity of CPH [5, 20, 21]. Since both, organic anions and cephalosporins accumulate preferentially in the proximal tubule cells of the renal cortex, homogenates of kidney cortex from CPH-treated rats were separated into different subfractions and their protein composition was analyzed. The results of SDS-gel electrophoresis of the cortical subfractions revealed significant alterations of the polypeptide pattern in the microsomal fraction, whereas in the lysosomal and cytosolic fractions no relevant changes occurred. The analysis of the polypeptide composition of the microsomal fraction showed that a polypeptide of molecular weight 44,000 was sig-

nificantly induced in the renal cortical microsomes from CPH-treated rats, whereas polypeptides in the molecular weight range 50–53,000 were depleted. It was of primary interest to determine whether the CPH-induced polypeptide of molecular weight 44,000 is a cytochrome P-450-protein. Induction experiments carried out with phenobarbital showed an increase of polypeptides in the 50–53,000 molecular weight region, but did not change the intensity of the 44,000 molecular weight polypeptide. The molecular weights of the renal polypeptides induced by phenobarbital treatment are in accordance with the molecular weights of cytochrome P-450 isoenzymes. Several cytochrome P-450 isoenzymes in microsomes from rat liver have been described [22, 23]. The molecular weights of these isoenzymes in discontinous SDS gel electrophoresis ranged from 45,000 to 60,000 [23–28]. In contrast to liver, little is known about cytochrome P-450 isoenzymes in the kidney. In microsomes from renal cortex of rabbits two cytochrome P-450 isoenzymes of molecular weights 57,000 and 58,000 were described [29]. In a previous paper we have shown that the decrease of polypeptides in the molecular weight region 50–53,000 after *in vivo* CPH-treatment of rats correlates with a decrease in the content of the renal cortical cytochrome P-450 isoenzymes [30]. These results and the data described above suggest that the renal microsomal polypeptide of apparent molecular weight 44,000 induced by CPH-treatment is not a known cytochrome P-450 isoenzyme.

Control experiments, where renal microsomes from control rats were incubated with CPH excluded the possibility that increase of the 44,000 molecular weight polypeptide is caused by a degradation of proteins of higher molecular weights by CPH. Hence, the increase in the amount of a polypeptide of molecular weight 44,000 in renal microsomes after CPH treatment is the result of protein induction. Solubilization experiments indicated that the CPH-induced polypeptide of molecular weight 44,000 is a peripheral rather than an integral membrane protein.

In preliminary experiments, acute treatment of rats with CPH (1200 mg/kg/d i.v.) for 3 days resulted in a significant decrease (<10% of control) in the ability of renal cortical slices to accumulate the organic cation tetraethylammonium (TEA) and to generate glucose. The decreases in TEA accumulation and gluconeogenesis, which are among other parameters used to estimate nephrotoxicity [31], correlated in a time- and dose-dependent manner with the induction of the 44,000 molecular weight microsomal polypeptide. Therefore, the induction of this polypeptide by CPH might be a sensitive parameter of CPH-induced nephrotoxicity. Similarly to CPH, various other xenobiotics such as carbon tetrachloride [32], or hypolipidemic drugs [33] have been shown to alter the protein composition of biological membranes. The function of the 44,000 molecular weight protein is not known at the present time. Preliminary data showed an increase in the enzymatic activities of drug-metabolizing enzymes such as the cytosolic GSH-*S*-transferases after treatment of rats with CPH. Therefore it seems possible that the CPH-induced polypeptide of apparent molecular weight 44,000 is a drug-metabolizing enzyme of the endoplasmic reticulum involved in the detoxification process of CPH.

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