METABOLISM AND NEPHROTOXICITY OF PHENACETIN AND SULFANILAMIDE

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The mechanisms of renal damage produced by sulfanilamide and phenacetin were studied. N-Hydroxyphenacetin, 4-hydroxylaminobenzenesulfonamide (4-HABSA) and p-aminophenol were established to be nephrotoxic metabolites. As well as N-hydroxylase activity of sulfanilamide, that of phenacetin in kidney microsomes of rats was increased about 4 times by pretreatment with 3, 4, 5, 3', 4'-pentachlorobiphenyl (PenCB) which is a potent inducer of cytochrome P-448. Parallel to this enzyme induction, pretreatment with PenCB enhanced the nephrotoxicity of phenacetin and sulfanilamide in rats. On the other hand, the formation of p-aminophenol from phenacetin was also confirmed in rat kidney in vitro.

These results suggested that 4-HABSA, N-hydroxyphenacetin and p-aminophenol formed in kidney play an important role in the renal damage produced by sulfanilamide and phenacetin.

**KEYWORDS — nephrotoxicity; phenacetin; sulfanilamide; N-hydroxylation; p-aminophenol; 3, 4, 5, 3', 4'-pentachlorobiphenyl

INTRODUCTION

Phenacetin had been widely used as an analgesic, but its world-wide use is now decreasing rapidly, because the prolonged use has been demonstrated to cause harmful side effects such as methemoglobinemia, renal papillary necrosis and non-obstructive pyelonephritis.1-30 These toxic effects have been suggested to be attributable to the active metabolites, N-hydroxyphenacetin4-6 or phenacetin-3, 4-epoxide, 9 which are formed by microsomal mixed-function oxidase systems. p-Aminophenol might also be a metabolite of phenacetin which has been found to be nephrotoxic in rats.6-8 Sulfanilamide is another nephrotoxic compound which has been shown to cause interstitial nephritis, polyarteritis, chronic pyelonephritis, and so on.9,10 Sulfanilamide is metabolized in liver microsomes to 4-hydroxylaminobenzenesulfonamide (4-HABSA) which is suggested to be a toxic metabolite.11

The present paper deals with the mechanism of nephrotoxicity produced by phenacetin and sulfanilamide on the basis of drug metabolism.

MATERIALS AND METHODS

Animals — Adult male Wistar rats weighing 150—200 g were used. Animals were allowed free access to water and food (CR-2, Tokyo Clea) ad libitum.

Materials — N-Hydroxyphenacetin (N-OH-phenacetin), mp 104°C and 4-hydroxylaminobenzenesulfonamide (4-HABSA), mp 140—141°C were synthesized according to the methods of Calder et al.5 and Yamamoto et al.12 respectively. 3, 4, 5, 3', 4'- Pentachlorobiphenyl (PenCB), mp 158.5°C was synthesized according to the method of Saeki et al.,13 nico- tinamide adenine dinucleotide phosphate reduced (NADPH) and 3-methylcholanthrene (3-MC) were purchased from Sigma Chemical Co. Ltd., and phenobarbital (PB) from Daiichi
Pharmaceutical Co. Ltd. All other compounds were obtained from commercial sources of reagent grade and purified by recrystallization before use.

*Treatments of Animals* — Aniline and its derivatives shown in Fig. 1 were dissolved in saline, and administered *i.p.* or orally at a dose of 1–2 mmol/kg. PenCB was dissolved in corn oil and injected *i.p.* into rats at a single dose of 0.5 mg/1.0 ml/kg. They were sacrificed 5, or after the injection. 3-MC and PB were dissolved in corn oil and saline, respectively, and injected *i.p.* once a day at a dose of 20 mg/2.0 ml/kg and 80 mg/1.25 ml/kg for 3 and 4 consecutive days, respectively. The rats were killed by decapitation 24 h after the last injection. Control animals were given either corn oil or saline.

*Toxicological Studies* — Urinary protein concentration, the activities of urinary enzymes and the concentration of blood urea nitrogen (BUN) were used as indicator of renal damage. Rats were kept in metabolic cages after the administration of drugs (1–2 mmol/kg, *i.p.* or orally) for 24 h and urine were collected. Protein concentration, creatinine concentration, N-acetylglucosaminidase (NAG) activity, and alkaline phosphatase activity in urine were determined by the usual methods. Blood was drawn by eye puncture under ether-anesthesia, and the serum was used for determination of BUN by urease-indophenol method. Furthermore, the renal damage was examined by the histological method with light microscopy and the enzymatic histochemical method for NAG.

*Preparation of Microsomes* — Microsomes of kidney and liver were prepared by the method previously described.

*Assay Methods* — 1) Sulfanilamide N4-hydroxylase activity was determined by the method previously described.

2) Phenacetin N-Hydroxylase Activity: The incubation mixture contained liver or kidney microsomes (6 mg of protein), 0.1 M Na-K phosphate buffer (pH 7.4), 6 µmol NADPH, 300 µmol of KF, and 9 µmol of phenacetin in a final volume of 3.0 ml. Incubation was carried out at 37 °C for 20 min with continuous shaking and terminated by adding 5 ml of ethyl acetate. Phenacetin and metabolites were extracted with ethyl acetate and ethyl acetate phase was evaporated under nitrogen atmosphere. The residue was dissolved in 100 µl of methanol, and an aliquot (20 µl) was used for the high performance liquid chromatography (HPLC) analysis, which was performed with a normal phase Radial Pak CN column (Water Associates Inc. Milford, MA). It was eluted with methanol containing 1 mM FeCl3, 1% acetic acid and 20% dimethylsulfoxide at a flow rate of 1.0 ml/min. The wavelength was set at 546 nm. The amount of N-hydroxyphenacetin was calculated from the standard curve by measuring the peak height.

3) Phenacetin Deethylase Activity: The incubation mixture contained liver or kidney microsomes (6 mg of protein), 0.1 M Na-K phosphate buffer (pH 7.4), 6 µmol NADPH, 3 µmol ethylenediaminetetraacetic acid (EDTA), 30 µmol MgCl2 and 6 µmol phenacetin in a final volume of 3.0 ml. Incubation was carried out at 37 °C for 20 min with continuous shaking and extracted after the termination by 5 ml of ethyl acetate. Ethyl acetate phase was evaporated under nitrogen atmosphere, the residue was dissolved in 0.1 ml of methanol, and an aliquot (10 µl) was used for the HPLC analysis by Radial Pak C18 column (Waters Associates Inc.). It was eluted with 0.1 M Na-K phosphate buffer (pH 7.4)–MeOH (6:4, v/v) at a flow rate of 1 ml/min, and at a detector sensitivity of 1.0.

4) Phenacetin Deacetylase Activity: The incubation mixture contained liver or kidney microsomes (6 mg of protein), 0.1 M Na-K phosphate buffer (pH 7.4), 6 µmol NADPH, 3 µmol EDTA, 30 µmol MgCl2 and 6 µmol phenacetin in a final volume of 3.0 ml. Incubation was carried out at 37 °C for 20 min with continuous shaking and terminated by adding 0.1 ml of 1.0 N NaOH and 5 ml of ethyl acetate. HPLC analysis was performed with the same solvent as that used in deethylolation reaction of phenacetin at a flow rate of 2 ml/min and at a detector sensitivty of 0.005.
5) Phenetidine Deethylase Activity: The incubation mixture contained liver of kidney microsomes (3 mg of protein), 0.1 M Na-K phosphate buffer (pH 7.4), 5 μmol NADPH, 3 μmol EDTA, 3 μmol phenetidine-HCl salt in a final volume of 3.0 ml. Incubation was carried out at 37 °C for 20 min with continuous shaking and terminated by adding 1 ml of 20% trichloroacetic acid, and proteins were precipitated from the incubation mixture. For the HPLC assay p-aminophenol was converted to p-hydroxybutyranilide by the method of Carpenter et al. To 1 ml of supernatant, 2 ml of 0.1 M Na-K phosphate buffer (pH 7.4) was added, followed by 50 μl of n-butyric anhydride. The solution was immediately mixed and allowed to stand at room temperature for 1 h. The entire solution was passed through a previously activated and washed C_{18} Sep Pak (Waters Associates Inc.) and the container was rinsed twice with 3–4 ml of H_{2}O which were also passed through the Sep Pak. p-Hydroxybutyranilide was then eluted with 4 ml of methanol, and after evaporation of the solvent, the residue was dissolved in 0.9 ml of 0.1 N NaOH and 0.1 ml of 1 M acetic acid. HPLC analysis was performed with a Radial Pak C_{18} column (Waters Associates Inc.) using a solvent of methanol–H_{2}O (1:1, v/v) at a flow rate of 1 ml/min.

6) Acetaminophen Deacetylation Activity: The incubation mixture contained 1 ml of liver or kidney 9000 × g supernatant, 0.1 M Na-K phosphate buffer (pH 7.4), 30 μmol MgCl₂, 9 μmol acetaminophen in a final volume of 3.0 ml. Incubation was carried out at 37 °C for 60 min with continuous shaking and terminated by adding 1 ml of 20% trichloroacetic acid. To measure p-aminophenol generated from acetaminophen, butyration and HPLC analysis were performed with the same method as that used in deacetylation reaction of phenetidine to p-aminophenol.

HPLC analysis was performed using a Waters Associates Liquid Chromatograph equipped with 6000 A pumps, a ultraviolet (UV) spectrophotometer with the wavelength set at 254 nm and a model U 6K injector. Cytochrome P-450 (448) content was determined according to the method of Omura and Sato using extinction coefficients of 91 cm⁻¹ mM⁻¹. Proteins in microsomes or 9000 × g supernatant were determined by the method of Lowry et al. with bovine serum albumin as a standard.

RESULTS

The Relationship between Renal Toxicity and

![Chemical Structures](attachment:image.png)

FIG. 1. Aniline and Its Derivatives Examined in Renal Toxicity
Molecular Structure

Renal toxicity of aniline and its derivatives including metabolites of phenacetin and sulfanilamide shown in Fig. 1 was examined by the following four methods; 1) the increase of urinary protein concentration 2) the increase of urinary enzyme activities 3) the increase of BUN level 4) the histological method using light microscopy, as described in materials and methods. As shown in Table I, urinary protein concentration, urinary NAG activity and BUN level were increased in rats administered with 4-HABSA and N-hydroxyphenacetin, but not by sulfanilamide and phenacetin treatment. At 48 h after i.p. administration of 4-HABSA (2 mmol/kg), the renal papillary necrosis (Fig. 2A) and a big cavity resulting from severe contraction of medulla (Fig. 2C) were observed.

In a series of aniline and aminophenol, p-aminophenol only produced severe renal damage, but aniline, o-aminophenol, m-aminophenol and p-chloroaniline did not (data not shown). Protein concentration, NAG activity and alkaline phosphatase activity in urine and BUN level were increased at 24 h after i.p. administration of p-aminophenol (1 mmol/kg) 3.0, 5.6, 3.3 and 2.8 times, respectively (Fig. 3). Many nuclei in proximal convoluted tubules also disappeared at this time (Fig. 2D, E), and furthermore in the histochemical study, NAG in proximal convoluted tubule was extremely diminished at 6 h after i.p. administration of p-aminophenol (1 mmol/kg) (Fig. 2F, G). From these results, it was concluded that p-aminophenol, 4-HABSA and N-hydroxyphenacetin were nephrotoxic.

The Correlation between Nephrotoxicity and Metabolism of Sulfanilamide or Phenacetin

a) N-Hydroxylation of Sulfanilamide and Nephrotoxicity — 4-HABSA formed from sulfanilamide in liver and kidney microsomes was identified by the method of silica gel thin layer chromatography (TLC) and HPLC as already reported. It was also found that both cytochrome P-450 (448) content and activity of N4-hydroxylase of sulfanilamide in kidney microsomes were markedly increased by the pretreatment with PenCB (about 4 times). In the present study, enhanced effect of PenCB pretreatment on the renal damage produced by sulfanilamide

### Table I. Effects of Drugs on Urinary Protein Contents, Urinary NAG Activity and BUN Level in Rats

<table>
<thead>
<tr>
<th>Drug</th>
<th>Route of administration</th>
<th>Dose (mmol/kg)</th>
<th>Number of animals</th>
<th>% control ± S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Urinary protein</td>
</tr>
<tr>
<td>Sulfanilamide</td>
<td>i.p.</td>
<td>1.0 × 1</td>
<td>3</td>
<td>99 ± 6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.0 × 2</td>
<td>4</td>
<td>61 ± 7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.0 × 1</td>
<td>4</td>
<td>79 ± 17</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.0 × 2</td>
<td>4</td>
<td>270 ± 170</td>
</tr>
<tr>
<td>4-Hydroxyaminobenzene-sulfonamide</td>
<td>i.p.</td>
<td>1.0 × 1</td>
<td>5</td>
<td>288 ± 78</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.0 × 1</td>
<td>4</td>
<td>267 ± 50</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.1 × 2</td>
<td>3</td>
<td>105 ± 22</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.1 × 1</td>
<td>5</td>
<td>214 ± 72</td>
</tr>
<tr>
<td>Phenacetin</td>
<td>Oral</td>
<td>1.0 × 1</td>
<td>5</td>
<td>98 ± 15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.1 × 1</td>
<td>5</td>
<td>153 ± 17</td>
</tr>
<tr>
<td>N-Hydroxyphenacetin</td>
<td>Oral</td>
<td>2.1 × 1</td>
<td>3</td>
<td>269 ± 51</td>
</tr>
</tbody>
</table>

See Materials and Methods for experimental procedures. Control values of urinary protein, urinary NAG activity, and BUN were as follows: 48.01 ± 7.24 mg/24 h, 45.57 ± 10.94 mmol of 4-methylumbelliferone liberated/mg creatinine/h, 14.16 ± 2.43 mg/dl serum. Each value represents a percent of control ± S.D.

a) Significant increase (p < 0.05).
FIG. 2. A. Renal Papillary Necrosis

Animals were killed 24 h after administration of 4-HABSA (2 mmol/kg, i.p.) and sections of kidney were stained with hematoxylin and eosin (H. & E., × 100).

B. Control Rat Kidney

C. Kidney Showing a Big Cavity Resulting from Severe Contraction of Medulla

Animals were killed 24 h after administration of 4-HABSA (2 mmol/kg, i.p.).

D. Control Rat (H. & E., × 100)

E. Renal Necrosis of Individual Cells of the Proximal Convoluted Tubules

Animals were killed 24 h after administration of p-aminophenol (1 mmol/kg, i.p.) and sections of kidney were stained with hematoxylin and eosin (H. & E., × 100).

F. The Histochemical Study for NAG in Proximal Convoluted Tubules

Sections of kidney of control rat were incubated at 37 °C for 30 min in the solution of 0.5 mM naphthol AS-BI N-acetyl-β-D-glucosaminide and hexazonium pararosaniline (× 100).

G. Animals were killed 6 h after administration of p-aminophenol (1 mmol/kg, i.p.) and sections of kidney were stained histochemically with the same method as the control (× 100).
was examined. Consequently, the increase of BUN level in rats receiving sulfanilamide (2 mmol/kg, i.p.) was enhanced about 1.5 times by pretreatment with PenCB as shown in Fig. 4. By this pretreatment, sulfanilamide induced necrosis of the proximal tubule, although any detectable change was not observed in the rats received sulfanilamide without the pretreatment (data not shown).

b) Metabolism of Phenacetin and Nephrotoxicity
— N-Hydroxylation reaction of phenacetin was studied with kidney and liver microsomes in rats pretreated with PB, 3-MC or PenCB by means of HPLC, using authentic N-hydroxyphenacetin as a standard. Phenacetin N-hydroxylase activity and cytochrome P-450 (448) content in kidney and liver microsomes were shown in Table II. In kidney microsomes, the PenCB pretreatment increased remarkably both cytochrome P-450 (448) content (about 2.5 times) and phenacetin N-hydroxylase activity (about 9 times). 3-MC also enhanced both parameters to a lesser extent, but PB did not affect at all. In liver microsomes, PB increased cytochrome P-450 content, but did not enhance N-hydroxylase activity. On the other hand, 3-MC and PenCB increased both parameters similarly as in kidney microsomes. These results suggest that phenacetin N-hydroxylation in liver and kidney microsomes is catalyzed by cytochrome P-448 which is induced selectively by 3-MC and PenCB. By Lineweaver–Burk plots of phenacetin

FIG. 3. Effect of p-Aminophenol on Urinary Protein, Urinary Enzyme Activities and BUN in Rats
(A) urinary protein (––), BUN (– - -); (B) urinary NAG activity (– – –), urinary alkaline phosphatase activity (– – –) were determined at various intervals after i.p. administration of p-aminophenol (1 mmol/kg).

The assay conditions were described in Materials and Methods. Each point is expressed as mean±S.E. of 3 experiments. a) Significantly different from the value at 0 time (p<0.05).
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$N$-hydroxylase activity in control rats, Michaelis constant ($K_m$) and maximum velocity ($V_{\text{max}}$) with kidney microsomes were found to be 3.571 mM and 0.056 nmol/mg protein/min, respectively.

FIG. 4. **Effect of Pretreatment with Various Inducers on Kidney Damage Produced by Sulfanilamide**

Doses of inducers are given in Materials and Methods. Urinary protein and BUN at 24 h after sulfanilamide (2 mmol/kg) treatment were determined by the methods of Lowry and the urease-indophenol method, respectively. Values are expressed as mean±S.D. of 4 or 6 experiments. a) Significantly different from the control ($p<0.05$).

□ untreated, ■ PenCB, □ 3-MC, □ PB.

**TABLE II. Effects of Various Inducers on Phenacetin N-Hydroxylase Activity and Content of Cyt. P-450 (448) in Kidney and Liver Microsomes of Rats**

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>$N$-OH-Phenacetin formed (pmol/mg protein/min)</th>
<th>Content of cyt. P-450 (448) (nmol/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Kidney</td>
<td>Liver</td>
</tr>
<tr>
<td>None</td>
<td>2.95±0.42</td>
<td>6.47±0.97</td>
</tr>
<tr>
<td>PB</td>
<td>2.53±0.42</td>
<td>6.56±0.63</td>
</tr>
<tr>
<td>3-MC</td>
<td>7.32±0.49</td>
<td>62.36±1.21</td>
</tr>
<tr>
<td>PenCB</td>
<td>26.42±1.49</td>
<td>113.62±16.47</td>
</tr>
</tbody>
</table>

See Materials and Methods for experimental procedures. Values are expressed as mean±S.D. of 3 or 5 experiments. a), b) Significantly different from the control ($a)p<0.05$, $b)p<0.01$).
ly. The $K_m$ and $V_{\text{max}}$ with liver microsomes 0.694 mM and 0.111 nmol/mg protein/min, were also determined to be respectively. Effects of the PenCB pretreatment on the renal damage produced by phenacetin was also studied. As the result, a significant increase of BUN level was observed by PenCB pretreatment (Fig. 5) and morphological change also supports this enhanced effect of PenCB on the renal damage caused by phenacetin (data not shown). Next, effects of PenCB pretreatment on the metabolic pathway of phenacetin to $p$-aminophenol in liver and kidney microsomes were studied. $p$-Aminophenol was formed from phenacetin via the following two routes; one is the deethylation of phenacetin (I), followed by the deacetylation of acetaminophen (IV). Another is the deacetylation of phenacetin (II) followed by the deethylation of phenetidine (III). For studies on the reactions I, II and III, liver and kidney microsomes were used together with NADPH as a cofactor, and for study of the reaction IV 9000 $\times g$ supernatant was used. As shown in Table III, the activities of reactions I and III in kidney microsomes were increased about 20 and 6 times, respectively by PenCB pretreatment. The activity of reaction IV in kidney was also enhanced 2.8 times. These results clearly indicate the production of $p$-aminophenol from phenacetin and its enhancement by PenCB pretreatment in the kidney as well as in the liver.

**DISCUSSION**

It has been known that sulfanilamide produces renal damage such as interstitial nephritis and polyarteritis. In the present study, the i.p. administration of 4-HABSA into rats caused severe renal damage (Fig. 2A, C). In the previous report, we proved for the first time the in vitro formation of 4-HABSA from sulfanilamide by using TLC and HPLC methods. Both $N^4$-hydroxylase activity of sulfanilamide and content of cytochrome P-450 (448) was enhanced by pretreatment with PenCB. In addition, apparent $K_m$ for $N^4$-hydroxylation of sulfanilamide in kidney and liver microsomes were found to be 0.83 and 1.54 mM, respectively. These values showed that cytochrome P-450 (448) in kidney microsomes has higher affinity for sulfanilamide than in liver microsomes. In the present study, we further proved that renal damage by sulfanilamide was enhanced by PenCB pretreatment. These results strongly suggested that the renal damage induced by sulfanilamide was mainly due to the 4-HABSA formation in kidneys.

On the other hand, hepatic or renal damage is caused by a large dose of phenacetin or acetaminophen, a main metabolite of phenacetin in man and experimental animals. The mechanisms for these toxicities are not yet clarified. Recent studies have provided indirect evidence in favor of $N$-acetyl-$p$-benzoquinoneimine being the active metabolite of acetaminohen which is produced immediately after the $N$-hydroxylation. Phenacetin also can be $N$-hydroxylated in liver microsomes and the possibility that $N$-hydroxylation was an important step for nephrotoxicity of phenacetin was examined. A single i.p. administration of
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N-hydroxyphenacetin caused renal damage, although phenacetin itself did not (Table I, Fig. 2). Furthermore we studied the N-hydroxylation of phenacetin with kidney and liver microsomes of rats by using HPLC method (Table II). The PenCB pretreatment increased both cytochrome P-450 (448) content and phenacetin N-hydroxylase activity in kidney microsomes, as well as in liver microsomes. But the Michaelis constant (K_m) for N-hydroxylation of phenacetin in kidney microsomes showed lower affinity compared with that in liver microsomes. However, part of N-hydroxylation of phenacetin could be also occurring in kidney, because compounds toxic to kidney are often found in large quantities in proximal convoluted tubules. In present study, we proved that renal damage by phenacetin was enhanced by PenCB pretreatment, which was exemplified by the increase of BUN level (Fig. 5) and morphological change.

Cytochrome P-450-mediated oxidative metabolism requiring NADPH and O_2 has been

TABLE III. Effects of PenCB Pretreatment on the Metabolic Pathway of Phenacetin

<table>
<thead>
<tr>
<th>Metabolic pathway</th>
<th>Control (nmol/mg protein/min)</th>
<th>PenCB (nmol/mg protein/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Liver</td>
<td>Kidney</td>
</tr>
<tr>
<td>I</td>
<td>0.788</td>
<td>0.068</td>
</tr>
<tr>
<td>II</td>
<td>0.224</td>
<td>0.017</td>
</tr>
<tr>
<td>III</td>
<td>0.124</td>
<td>0.034</td>
</tr>
<tr>
<td>IV</td>
<td>0.129</td>
<td>0.051</td>
</tr>
</tbody>
</table>

See Materials and Methods for experimental procedures. Values are expressed as mean of two experiments.
believed to be the sole pathway for the toxic metabolic activation of acetaminophen. However, another metabolic pathway for the activation of acetaminophen was found in rabbit renal inner medulla by Mohandas et al. and Anderson et al. This alternative pathway was recognized as cooxidation of acetaminophen mediated by prostaglandin endoperoxide synthetase, requiring arachidonic acid and O₂. The N-hydroxylation of phenacetin in the presence of rat kidney medulla and cortex by arachidonic acid-dependent cooxidation was therefore examined according to the method of Mohandas. However, N-hydroxyphenacetin was detected neither in the rat kidney medulla nor cortex. Cytochrome P-450, which is responsible for the metabolism of those drugs, is localized only in the proximal tubules. Therefore, the damage of proximal tubules may be caused by toxic metabolites from phenacetin and sulfanilamide.

A number of compounds related to phenacetin have been shown to cause necrosis of the proximal convoluted tubules of the kidney when they were administered in a single intravenous dose. p-Aminophenol has been shown to be a potent nephrotoxin among these compounds. We examined, therefore, the metabolic pathway of phenacetin to p-aminophenol via two routes in liver and kidney microsomes or 9000 × g supernatant as shown in Table III. When the effects of pretreatment with PenCB on each reactions were examined, the reactions I, III and IV were increased in kidney and liver microsomes. These results indicate that p-aminophenol is formed also in kidney from phenacetin. Very recently, p-aminophenol has also been shown by other groups to occur in a significant amount as a metabolite of both phenacetin and acetaminophen. Therefore, p-aminophenol formed in kidneys was suggested to play an important part as well as N-hydroxyphenacetin in renal damage produced by phenacetin.

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