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Metabolism of Drugs. LXIV.[1] Species Differences of Metabolism of Phenacetylurea

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In our previous papers, it was shown that phenacetylurea administered to rabbits is metabolized to 3-methoxy-4-hydroxyphenacetylurea, but in mice it is not converted to this unique metabolite.

The present study revealed that the above metabolite is also excreted as one of the major metabolites in the urine of guinea pigs and a human after doses of phenacetylurea, as well as rabbits, whereas rats apparently do not follow to this pathway.

Although in these species of animals and in a human the metabolic patterns of phenacetylurea were somewhat different each other, the drug was metabolized by two main pathways, hydroxylation of aromatic ring and hydrolysis of ureide group in all species examined.

The identification and determination of the metabolites in animal species were facilitated by use of ^{14}C-labeled phenacetylurea.

In the previous paper of this series,[3] it was shown that in rabbits phenacetylurea is metabolized along two different pathways; one is successive hydroxylation at the 4-position and then 3-position of the benzene ring, followed by methylation of the 3-hydroxyl group to 3-methoxy-4-hydroxyphenacetylurea, and the other is hydrolysis of the ureide group to phenylacetic acid and subsequent conjugation with glycine to phenaceturic acid. Among these metabolites, 3-methoxy-4-hydroxyphenacetylurea was the most attractive one, because the *in vivo* formation of such a metabolite has never been reported in the metabolism of foreign compounds possessing a aromatic ring, except this example. Such an aromatic compound is usually metabolized by hydroxylation and subsequent conjugation.

In the following study using mice,[1] however, it was found that there was a remarkable species difference of the metabolism of phenacetylurea between rabbits and mice. Thus, the unique metabolite in rabbits, 3-methoxy-4-hydroxyphenacetylurea could not be detected at all in urine of mice, although the latter animals also excreted 4-hydroxylated metabolite.

The present investigation was undertaken in order to confirm this species difference more extensively using rats, guinea pigs and human. To facilitate the experiment ^{14}C-phenacetyl urea labeled in the acyl carbonyl group was utilized in the present study and the results were quantitatively compared each other. The metabolism in rabbits was also reexamined to obtain quantitative information by use of this radioactive drug. Further experiments were undertaken to investigate the *in vitro* conversion of phenacetylurea to the 4-hydroxylated metabolite, and also to determine the pharmacological activity of the phenolic metabolites and of several related compounds.

Experimental

Materials——^{14}C-Phenacetylurea, labeled in the acyl carbonyl was prepared according to the method described in the previous paper.[1] PPO (2,5-diphenyloxazole) and POPOP (1,4-bis-2-(5-phenyloxazolyl)-

1) Part LXIII: K. Tatsumi, S. Yoshihara, H. Yoshimura and H. Tsukamoto, *Biochem. Pharmacol.*, **18**, 365 (1969)
2) Location: *Katakasu, Fukuoka.*
3) K. Tatsumi, H. Yoshimura and H. Tsukamoto, *Biochem. Pharmacol.*, **16**, 1941 (1967).

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benzene) were purchased from Wako Pure Chemical Industries, Ltd., Osaka. Nonlabeled phenacetylurea was donated by Dainippon Pharmaceutical Inc., Osaka. The β-glucuronidase preparation was obtained from preputial glands of adult female rats and its activity (116000 units/ml) was determined using *p*-nitrophenyl-β-D-glucuronide as the substrate. The animals used were male rats (about 180 g), guinea pigs (about 380 g) and rabbits (about 2.4 kg).

Synthesis of 4-Methoxy-, 3,4-Dimethoxy- and 3,4,5-Trimethoxyphenacetylurea——4-Methoxy-, 3,4-dimethoxy- and 3,4,5-trimethoxyphenylacetic acid were converted to the corresponding methyl ester by treatment with CH_2N_2 by the usual method, and the esters were condensed with urea by a method similar to that used for 4-hydroxyphenacetylurea, which was described in the previous paper.[3] The resulting ureides were recrystallized from EtOH as colorless crystals. 4-Methoxyphenacetylurea: mp 210—213°. *Anal.* Calcd. for $C_{10}H_{12}O_3N_2$: C, 57.68; H, 5.81; N, 13.46. Found: C, 58.13; H, 5.93; N, 13.54. 3,4-Dimethoxyphenacetylurea: mp 189—191°. *Anal.* Calcd. for $C_{11}H_{14}O_4N_2$: C, 55.45; H, 5.92; N, 11.76. Found: C, 55.69; H, 6.35; N, 11.95. 3,4,5-Trimethoxyphenacetylurea: mp 194—197°. *Anal.* Calcd. for $C_{12}H_{16}O_5N_2$: C, 53.72; H, 6.01; N, 10.44. Found: C, 53.68; H, 6.05; N, 10.51.

Administration of Phenacetylurea——^{14}C-Phenacetylurea (specific activity, 0.25 μCi/mg) was mixed with pure nonlabeled compound to obtain specific activities of 0.1, 0.05 and 0.02 μCi per mg in the experiments using rats, guinea pigs and rabbits prior to use, respectively. These were administered orally to the animals in a dose of 100 mg/kg as a suspension in an appropriate volume of 10% gum arabic unless otherwise stated. The animals were fasted overnight prior to use. After medication, each animal was housed in a individual metabolic cage. Food and water were available at all times. A patient was given in a single dose of 1 g of nonlabeled phenacetylurea.

Extraction of Metabolites——The urine from each species of animals, which received ^{14}C-phenacetylurea, was adjusted to pH 1 with conc. HCl and then extracted four times with equal volume of AcOEt by shaking mechanically. The urine from a patient given nonlabeled phenacetylurea was adjusted to pH 2.5 with 20% H_2SO_4, followed by the continuous extraction with AcOEt for 20 hr.

Paper and Thin-Layer Chromatography——Paper chromatography was carried out by the ascending technique with filter paper (Toyoroshi No. 51A, Toyoroshi Co., Tokyo). Thin-layer chromatography was carried out by use of silicagel plates, 0.25 mm thick (Kiessel gel G, Merck) which was activated at 110° for 60 min. The solvent systems used were 1) *n*-BuOH saturated with 1.5N NH_4OH; 2) benzene–$CHCl_3$–acetic acid (2:1:1); 3) *n*-BuOH–5% NH_4OH (10:1). Phenolic compounds were visualized by spraying 0.2% solution of diazotized sulfanilic acid in 10% Na_2CO_3 solution. Phenacetylurea was also visualized by spraying successively 5% sodium nitroprusside solution and 30% NaOH solution. Bromthymol blue reagent (40 mg of bromthymol blue in 100 ml of alkaline EtOH) and a slightly modified Ehrlich reagent[4] were used for detection of phenylacetic acid and phenaceturic acid, respectively.

Radioisotope Methods of Analysis——The radioactivity of all samples was measured using a Beckman liquid scintillation spectrometer (Model DPM-100$^{T.M.}$). Urine was filtered through filter paper (Toyoroshi No. 2, Toyoroshi Co., Tokyo). Aliquots of urine (0.1 to 0.4 ml) were counted in a *p*-dioxane phosphor consisting of 60 g naphthalene, 4 g PPO, 0.2 g POPOP, 100 ml methanol, 20 ml ethyleneglycol and *p*-dioxane to make 1 liter. An internal standard of ^{14}C-toluene was used to determine counting efficiency.

Radioactivity of thin-layer chromatograms was qualitatively determined by using a thin-layer radiochromatogram scanner (Japan Radiation & Medical Electronics, Inc., Tokyo). Radioactivity on paper chromatograms was determined by cutting the chromatogram into 1 cm strips, placing these in vials containing 10 ml of a toluene phosphor (4 g PPO and 0.1 g POPOP in 1 liter of toluene) and counting the radioactivity in a liquid scintillation spectrometer.

Preparation of Tissue Samples——Adult male rabbits were killed and exanguinated, and the livers were removed immediately. Liver slices were prepared by free hand method with razor blade. The livers were homogenized with 2 volumes of cold 1.15% KCl solution in a Potter-Elvehjem homogenizer and the homogenate was centrifuged at 9000*g* for 20 min. To prepare the microsomal fraction, the 9000*g* supernatant was centrifuged at 105000*g* for 1 hr. The homogenate of the various organs, kidney, adrenal, spleen, small intestine, heart, lung or pancreas of a rabbit and liver homogenates of mice were prepared similarly. In the study using phenylalanine hydroxylase system, 0.01M acetic acid extract (18000*g* supernatant) and crude cofactor were prepared from rat liver according to the method reported by Kaufman.[5]

Enzyme Assay——Phenacetylurea (or acetanilide) was incubated in Tris–phosphate buffer, pH 8.5 for 1 hr at 37° using metabolic incubator. The incubation mixtures consisted of (1) 20 μmoles of substrate, 1 g of liver slice and 10 ml of the buffer; (2) 1 μmole of substrate, 4 μmoles of nicotinamide, 2 μmoles of $MgCl_2$, 0.3 ml of homogenate equivalent to 1 g liver and the buffer to make a final volumes of 1.5 ml; (3) 2 μmoles of substrate, 1 ml of 9000*g* supernatant equivalent to 0.33 g of liver and the buffer to make a final

4) G.W. Gaffney, K.S. Schreier, N. Diferrante and K.I. Altman, *J. Biol. Chem.*, **206**, 695 (1954).
5) S. Kaufman, *J. Biol. Chem.*, **226**, 511 (1957); *Idem, ibid.*, **230**, 931 (1958).
6) B.B. Brodie and J. Axelrod, *J. Pharmacol. Exptl. Therap.*, **94**, 22 (1948).
7) S. Udenfriend and J.R. Coopper, *J. Biol. Chem.*, **196**, 227 (1952).

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volume of 3.5 ml; (4) 10 μmoles of substrate, 2.5 μmoles of NADPH, 2 ml of microsomal fraction equivalent to 1 g of liver and the buffer to make a final volume of 8 ml. In the study using phenylalanine hydroxylase system, 2 μmoles of phenacetylurea (or phenylalanine) was incubated for 1 hr at 37° in a mixture which contained 0.3 μmoles of NADPH, 0.2 ml of crude cofactor, 0.01M acetic acid extract equivalent to 0.025 g of liver, 0.1 ml of 1.0M potassium phosphate buffer of pH 6.8 and water to make a final volume of 1 ml.

The phenolic metabolites, which would be derived from phenacetylurea, were examined by thin-layer chromatography after extraction with AcOEt. *p*-Acetylaminophenol formed from acetanilide was assayed by the method of Brodie, *et al.*[6] Tyrosine formed from phenylalanine was determined by that of Udenfriend, *et al.*[7]

Results

Excretion of Radioactivity

Table I shows the percentage of the radio-activity appearing in the urines of rats, guinea pigs and rabbits during 48 hr. The rabbit urine contained the greatest radioactivity. Rats and guinea pigs do not differ markedly from mice[8] in their urinary excretion of radioactivity.

Table I. Percent Recovery of ^{14}C in Urine of Rats, Guinea Pigs and Rabbits after Oral Administration of ^{14}C-Phenacetylurea

Time (hr)	Rats (4)	Guinea pigs (2)	Rabbits (2)
0—24	51.1	46.0	81.7
24—48	12.2	9.9	5.7
Total	63.3	55.9	87.4

Figures in parentheses represent the number of animals.

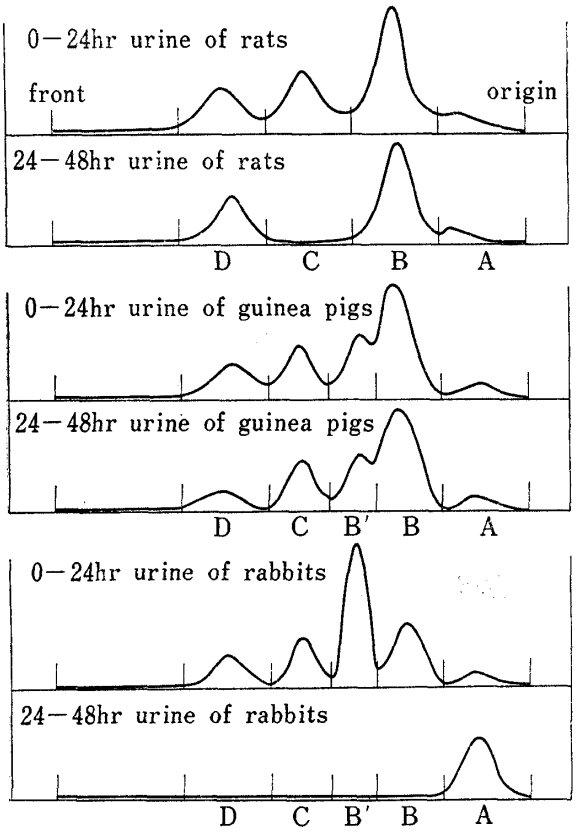

Fig. 1. Radioactive Scan of Thin-layer Chromatogram of Ethyl Acetate Extract from Urine of Rats, Guinea pigs, and Rabbits after Administration of ^{14}C-Phenacetylurea

Separation and Detection of the Urinary Metabolites in Rats

The AcOEt extracts of the first day and the second day urines from rats were concentrated to a small volume and submitted to thin-layer chromatography using system 2 (Fig.1). Peak A,B,C, and D were eluted with MeOH and rechromatographed separately on filter paper using system 1.

By this treatment peak A was further separated into two radioactive peaks, *Rf* 0.05 and 0.23. However, none of these *Rf* values was in agreement with those of known compounds, and therefore these are tentatively designated as unknown I and II, respectively. Further investigation of these spots has not been performed. Paper chromatography of peak B yielded two radioactive peaks with *Rf* values of 0.31 and 0.74. These two peaks correspond to those of the authentic phe-

8) Mice excreted about 60% the radioactivity in 48 hr urine.

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naceturic acid and 4-hydroxyphenacetylurea, respectively. Peak C exhibited two radioactive peaks on paper with *Rf* values of 0.52 and 0.93, which were identified as phenylacetic acid and phenacetylurea, respectively. The redioactive zone of peak D yielded two radioactive peaks, identical with those of peak C, having *Rf* values of 0.52 and 0.93. Comparing the radioactivity of these two metabolites in peak C and D, the activity of phenacetylurea was higher in peak C, whereas that of phenylacetic acid higher in peak D.

The aqueous phase of first day urine remained after AcOEt extraction was concentrated to a small volume *in vacuo* and submitted to thin-layer chromatography using system 2 (Fig.2). The radioactive peak close to the origin was eluted from the silicagel with MeOH, and the eluate was evaporated to dryness *in vacuo*. The residue was treated with β-glucuronidase in 0.1M acetate buffer (pH 4.5) at 37° for 24 hr and then rechromatographed on thin-layer plate using the same solvent system. The radioactive scan of the chromatogram revealed the presence of a new peak, in addition to the original peak (Fig. 3). This new peak was, however, also obtained by control experiment without β-glucuronidase, and therefore it was considered that the material corresponding to the new peak might be spontaneously produced from the original one during the course of treatment. From above results, it was concluded that the aqueous phase contained no significant amount of O-glucuronide.

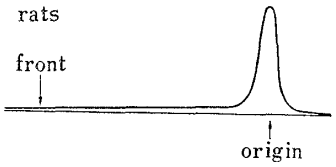

Fig. 2. Radioactive Scan of Thin-layer Chromatogram of the Aqueous Phase of 24 hr Urine

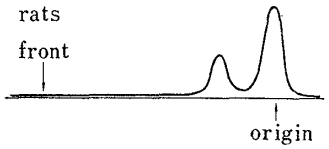

Fig. 3. Radioactive Scan of Thin-layer Chromatogram after Hydrolysis of the Peak seen in Fig. 2

Separation and Detection of the Urinary Metabolites in Guinea Pigs and Rabbits

The AcOEt extracts of the first day and the second day urine from guinea pigs and rabbits were also examined by the thin-layer chromatography same as that for rat. As seen from the radioactive scan of the chromatogram (Fig. 1), metabolic patterns of both species were very similar and had extra peak B′ as compared with that of rats. Peak A was detected in a considerable amount only in the second day urine of rabbits. The further investigation of this peak has not been undertaken and remained unidentified. Peak B, B′, C, and D were eluted with MeOH and rechromatographed on thin-layer plates using system 1. By this treatment peak B yielded two radioacitve peaks with *Rf* values of 0.24 and 0.73 which correspond to phenaceturic acid and 4-hydroxyphenacetylurea, respectively. Peak B′, C and D exhibited only one distinct peak with *Rf* values of 0.67, 0.68 and 0.38 after rechromatography, and identified as 3-methoxy-4-hydroxyphenacetylurea, unchanged compound and phenylacetic acid, respectively.

Determination of Metabolites in the Urines of Rats, Guinea Pigs and Rabbits

The metabolite composition of the first day and the second day urine of rats, guinea pigs and rabbits dosed with ^{14}C-phenacetylurea was investigated. Each AcOEt extract from these animals was evaporated to dryness *in vacuo*. The residue was redissolved in AcOEt to make 25 ml, of which 0.2 ml was pipetted and counted in a toluene phosphor. The aqueous phase remained after AcOEt extraction was diluted to 25 ml with distilled water, of which 0.2 ml was also counted in a dioxane phosphor. Table II shows the distribution of the radioactivity between ethylacetate extract and aqueous phase in each animal. The radioactivity in both fractions of rabbits was approximately even. Furthermore, it was also suggested that the excretion of polar metabolites in rats decreased in the second day (24 to 48 hr urine) as

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Table II. The Distribution of the Radioactivity between AcOEt Extract and Aqueous Phase in Various Animals

		Mouse[a]	Rat	Guinea pig	Rabbit
0—24 hr Urine	AcOEt extract	59.3	73.6	77.1	57.1
	Aqueous phase	40.7	26.4	22.0	42.9
24—48 hr Urine	AcOEt extract	—	81.9	66.5	42.1
	Aqueous phase	—	18.1	33.5	57.9

a) Data were quated from previous paper.[1]

compared to the first day (0 to 24 hr urine), while these excretion in guinea pigs and rabbits rather increased.

Table III shows the results of the quantitative determination of each metabolite in urines of rats, guinea pigs and rabbits. The amount of the metabolites in human urine was estimated approximately by thin-layer chromatography. The unique metabolite, 3-methoxy-4-hydroxyphenacetylurea was found as one of the main metabolites in the urines of guinea pigs, rabbits and human. On contrary any detectable amount of this metabolite was not present in the urines of rats, similar to mice.[1]

Table III. The Metabolites of Phenacetylurea in Various Animals and Human

Metabolites	Mouse[a]	Rat	Guinea pig	Rabbit	Human
Phenacetylurea	20.8	22.6	22.2	8.6	++++
4-Hydroxyphenacetylurea	14.5[b]	5.4	14.1	6.8	+++
3-Methoxy-4-hydroxyphenacetylurea	0	0	10.0	20.7	+
Phenaceturic acid	2.4	25.5	7.6	9.4	—
Phenylacetic acid	9.3	11.8	14.8	11.6	—
4-Hydroxyphenylacetic acid	0.5[c]	0	0	0	++
Unknown	52.5	34.7	31.3	42.9	—

a) Data were quated from previous paper.[1] b) free+glucuronide c) Excreted as glucuronide.

Separation and Detection of Metabolites in Human Urine

The AcOEt extract of the 24 hr urine of a patient received nonlabeled phenacetylurea was evaporated to dryness *in vacuo* to leave brown gum (2.3 g). It was dissolved in $CHCl_3$ containing a small volume of MeOH and then submitted to column chromatography (40 g silicagel was packed) according to the procedure described in previous paper.[3] The column was eluted stepwise with $CHCl_3$, $CHCl_3$–MeOH (99:1), $CHCl_3$–MeOH (98:2) and $CHCl_3$–MeOH (97:3). Each fraction was examined by thin-layer chromatography using system 2.

As the result, unchanged phenacetylurea was obtained from the $CHCl_3$ fraction as colorless crystals, which showed mp 212—216° by recrystallization from EtOH. Its UV and IR spectra were entirely identical with those of the authentic phenacetylurea. From the fraction of $CHCl_3$–MeOH (98:2), the spot having the same *Rf* value as that of the authentic 3-methoxy-4-hydroxyphenacetylurea (*Rf* 0.49) was detected. The area corresponding to this spot was eluted with MeOH from silicagel and subsequently rechromatographed on thin-layer plate using system 3. In this system, the compound (*Rf* 0.45) had also the same mobility as that of the authentic sample. The UV spectrum of the extract from the area of *Rf* 0.45 was identical with that of the authentic 3-methoxy-4-hydroxyphenacetylurea. Alkaline hydrolysis of this extract gave homovanillic acid, identified by thin-layer chromatography using the two solvent systems described above. By the same thin-layer chromatography 4-hydroxyphenacetylurea was detected in the fractions of $CHCl_3$, $CHCl_3$–MeOH (99:1), $CHCl_3$–MeOH (98:2) and $CHCl_3$–MeOH (97:3), and 4-hydroxyphenylacetic acid in the fraction of $CHCl_3$–

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MeOH (98:2). Also the UV spectra of these two metabolites were identical with the corresponding authentic samples, respectively.

Metabolism of Phenacetylurea administered intraperitoneally as compared to That administered orally in Rabbits

Phenacetylurea was given orally to one male rabbit and intraperitoneally to another in a dose of 200 mg/kg, respectively. The third rabbit was used as a control. The 24 hr urine of each rabbit was used for detection of phenolic metabolites as follows; It was extracted continuously at pH 2.5 with AcOEt for 20 hr. The AcOEt extract was evaporated to dryness *in vacuo*. The residue was redissolved in 30 ml of AcOEt, filtered and shaken four times with 10 ml of saturated sodium bicarbonate solution. The AcOEt phase was evaporated to dryness *in vacuo*. The residue was submitted to silicagel column chromatography same as the extract of human urine. Each fraction was examined by thin-layer chromatography using the two solvent systems, 2 and 3. Consequently 3-methoxy-4-hydroxyphenacetylurea was detected from the fraction of $CHCl_3$–MeOH (97:3) and 4-hydroxyphenacetylurea from the fraction of $CHCl_3$–MeOH (95:5) in both orally and intraperitoneally dosed animals. The structures of these metabolites were reconfirmed by the measurement of their UV spectra. On the other hand, the urine sample from a control rabbit did not give any spots corresponding to the metabolites.

The *In Vitro* Study of Hydroxylation of Phenacetylurea

Phenacetylurea was incubated with slices, homogenates, 9000 ***g*** supernatants or microsomes of rabbit liver under the conditions described under "Experimental". None of these tissue preparations, however, could not hydroxylate the drug, whereas acetanilide was readily hydroxylated in these systems. Attempts to demonstrate ring hydroxylation of phenacetylurea using the homogenate of various organs of rabbit or the liver homogenate of mouse were also unsuccessful. The possibility of ring hydroxylation of phenacetylurea by phenylalanine hydroxylase system was investigated by the method of Kaufman. However, the phenolic metabolites could not be detected at all in the extract of the reaction mixture, although a distinct formation of tyrosine was observed in the incubation of phenylalanine with the same liver preparation.

Preliminary Test of Pharmacological Action of the Metabolites and Its Related Compounds

According to the method of Everett and Richards,[9] the anticonvulsant effect of the metabolites, 4-hydroxyphenacetylurea and 3-methoxy-4-hydroxyphenacetylurea, and its related compounds, 4-methoxyphenacetylurea, 3,4-dimethoxyphenacetylurea and 3,4,5-trimethoxyphenacetylurea was examined using male mice of the CF-1 strain weighing about 20 g. The oral administration of these compounds (500 mg/kg) did not prevent, after 1 hr, the convulsion with supramaximal electroshock, strychnine (1.5 mg/kg, *s.c.*) and metrazol (100 mg/kg, *s.c.*), while phenacetylurea (500 mg/kg, *p.o.*) and meprobamate (500 mg/kg, *p.o.*) were markedly effective against the convulsions.

Discussion

In the previous papers, it was reported that an interesting metabolite, 3-methoxy-4-hydroxyphenacetylurea was isolated as the main metabolite from the urine of rabbits which received phenacetylurea,[3] however in mice this metabolite could not be detected at all under the same experimental condition.[1]

The present investigation was therefore undertaken to examine whether this metabolite confines only to rabbits or not, using rats, guinea pigs and human, and also to elucidate how

9) G.M. Everett and R.K. Richard, *J. Pharmacol. Exptl. Therap.*, **106**, 303 (1952).

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the metabolic pattern would change among these species. Regarding the metabolism of phenacetylurea in rats, Asher, *et al.*, reported that a minimum of three spots present on paper chromatograms, however, they did not identify any of the metabolites. In the present *in vivo* study it was found that the urinary products of rats include unchanged compound, 4-hydroxyphenacetylurea, phenaceturic acid, phenylacetic acid, as well as several other unidentified metabolites. However, rats apparently did not convert phenacetylurea to 3-methoxy-4-hydroxy metabolite.

Everett and Richards reported that no appreciable quantities of phenacetylurea were detectable in the human urine after a single dose of 2g of the drug. The present study showed that a patient given 1 g of phenacetylurea excretes at least 3-methoxy-4-hydroxyphenacetylurea, 4-hydroxyphenacetylurea and 4-hydroxyphenylacetic acid, in addition to the unchanged compound. In these metabolites, 4-hydroxyphenylacetic acid is known also as the intermediary metabolite, however it seems that at least a part of it must be attributable to the given drug considering the amount excreted. 3-Methoxy-4-hydroxyphenacetylurea was also found in the urine of guinea pigs after dose of phenacetylurea. From above results, it is concluded that double hydroxylation and subsequent methylation on aromatic ring occurs not only in rabbits, but also in guinea pigs and human, and thus the metabolic pattern of phenacetylurea in guinea pigs, rabbits and human are similar each other, but differ from that of rats which are rather resemble that of mice.

Among the various metabolites of phenacetylurea, 4-hydroxyphenacetylurea and 3-methoxy-4-hydroxyphenacetylurea can be considered to constitute the products of the ring hydroxylation pathway. Phenylacetic acid and phenaceturic acid arises from the hydrolysis pathway. As seen in Table III which shows the percentage of metabolites excreted into the urine of each species, both ring hydroxylation and hydrolysis of ureide group are the major pathways in most species, and only in the rats the hydrolysis pathway is prominent. The mechanism of the formation of 3-methoxy-4-hydroxy metabolite from phenacetylurea is uncertain at the present time, although it can be assumed that it is produced *via* successive hydroxylation and methylation, same to the formation of metanephrine or 2-methoxyestrone. In order to establish this pathway, the first attempt was made to show the *in vitro* system which is capable to *para*-hydroxylate phenacetylurea, using various liver preparations of mice and rats. However, neither drug metabolizing enzyme systems nor phenylalanine hydroxylase system in these preparations could be hydroxylated phenacetylurea.

Finally, in order to examine any possibility that the production of 4-hydroxyphenacetylurea and 3-methoxy-4-hydroxyphenacetylurea from phenacetylurea might be attributed to intestinal bacteria, additional experiments were performed, and this was essentially excluded, since phenacetylurea, even when administered intraperitoneally, was metabolized to these compounds. The same result was obtained also in mice.

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