The comparative metabolism of phenacetin and N-acetyl-p-aminophenol in man, with particular reference to effects on the kidney

The metabolism of phenacetin and N-acetyl-p-aminophenol (APAP) was investigated in conjunction with studies of renal tubular cell excretion. A significant increase in renal tubular cell excretion was observed in 2 of 9 healthy volunteers receiving a total of 13.5 Gm. of phenacetin in 5 days and in 1 of 8 volunteers receiving the same dose of APAP. Following oral doses of 1.8 Gm., the maximum plasma concentrations of phenacetin varied from 1.3 to 24.8 μg per milliliter, and there were often marked differences in the same individual during repeated studies. There was less variation in the plasma concentrations of free APAP following administration of phenacetin or APAP and the half-life of both drugs in the plasma was relatively constant in a given individual. Approximately 78 per cent of the dose of phenacetin and 85 per cent of the APAP was recovered as free and conjugated APAP in the urine in 24 hours.

Many pigmented bands were observed on thin layer chromatograms of hydrolyzed urine extracts from subjects receiving phenacetin. In contrast, negligible amounts of pigment were present when APAP was taken. It was not possible to demonstrate differences in the absorption, excretion, or metabolism of phenacetin or APAP in volunteers showing a marked renal tubular cell response compared with those who did not.

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During the last 15 years there have been numerous reports relating renal papillary necrosis and nonobstructive pyelonephritis (chronic interstitial nephritis) to the abuse of analgesic mixtures. Phenacetin is alleged to be the nephrotoxic agent since it has been present in the majority of analgesic preparations implicated. However, other potentially nephrotoxic drugs such as salicylates, pyrazolone derivatives, and caffeine have always been taken concurrently by patients reported to have analgesic-induced

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nephropathy. Renal lesions comparable to those of human pathology have been difficult to produce in experimental animals treated with large doses of phenacetin.

Measurement of urinary renal tubular cell excretion has been employed as a sensitive indicator of renal tubular injury.[1, 11] In previous studies, a striking increase in renal tubular cell excretion was regularly observed in healthy volunteers receiving 3.6 Gm. of aspirin daily for 5 days. Only 4 of 28 volunteers given 3.6 Gm. of phenacetin daily responded with a marked increase in renal tubular cell counts, and little or no effect was observed during treatment with the same dose of N-acetyl-p-aminophenol (APAP, acetaminophen, paracetamol), even in volunteers known to respond to phenacetin.[17, 18]

Since phenacetin is rapidly and extensively metabolized to APAP,[3, 9] either phenacetin itself or metabolites other than APAP seem likely to be responsible for the observed effects on renal tubular cell excretion. This response only occurred in a minority of normal individuals receiving phenacetin, and it seemed possible that this susceptibility could be explained by individual variation in the metabolism of phenacetin. The comparative metabolism of phenacetin and APAP was, therefore, studied in relation to renal tubular cell excretion in normal adult volunteers.

Methods

Differential renal tubular cell, red blood cell, and leukocyte excretion rates were measured in 17 healthy adult volunteers during 5 day control and treatment periods as described previously.[17] Nine volunteers received phenacetin* and 8 received APAP; none had taken any drugs during the preceding 6 weeks.

On the first treatment day the fasting volunteers took 900 mg. of phenacetin or APAP in gelatine capsules at 8:30 A.M. (zero time), followed by a further 900 mg. at 9:00 A.M. Urine samples were obtained for cell counts and then pooled in a 24 hour collection. Blood (20 ml.) was taken into tubes containing heparin at 0, 1, 2, 4, 6, 8, and 24 hours and the plasma separated immediately by centrifugation. The same procedure was carried out on the fifth day. Urine and plasma samples were stored frozen until the time of analysis.

A dose of 3.6 Gm. of phenacetin or APAP was given in 4 equal parts on Days 2 and 3; the dosage schedule on the fourth day was the same except that the evening dose was omitted. Thus, 24 hour urine collections and serial blood samples were obtained following ingestion of 1.8 Gm. of phenacetin or APAP on the first and fifth days. A total of 3.6 Gm. of drug was taken on the second and third days and 2.7 Gm. on the fourth day.

Phenacetin and free APAP in plasma were estimated in duplicate by the diazotization method of Brodie and Axelrod,[2, 3] and free and conjugated APAP in urine was assayed by the indophenol method of Welch and Conney.[22] Thin layer chromatography of organic solvent extracts of glusulase-treated* urine was carried out as described by Klutch and colleagues.[13] One-tenth per cent of a 24 hour urine sample was diluted to 3.25 ml. with water. To this was added 2.0 ml. of 0.2M sodium acetate buffer (pH, 5.0) and 0.75 ml. of glusulase. This mixture was incubated for 4 hours at 37° C. with gentle shaking. The mixture was extracted with 30 ml. of methylene dichloride, and a 20 ml. portion was evaporated to dryness under nitrogen. The residue was dissolved in a small volume of methanol or acetone and chromatographed. Thin layer plates (20 by 20 cm.) were prepared with the use of aluminum oxide GF_{254}, and the chromatograms were developed with the upper phase of benzene: water:acetic acid (2:1:2) or the lower phase of ethylene dichloride:methanol:

*Phenacetin (Mallinckrodt, Control No. WNBT) Contained less than 0.01% p-chloracetin. APAP was obtained from Direct Laboratories, Inc., Buffalo, N. Y. Lot 4-131B.

*Glusulase (Endo Laboratories, Inc.) contains 100,000 Fishman units of beta glucuronidase and 50,000 units of arylsufatase per milliliter and other unknown hydrolytic enzymes.

water:acetic acid (4:1:1:4) mixtures. The latter solvent system was employed for the examination of pigments in the urine. The plates were examined under visible light, under ultraviolet light, and after spraying with Pauly reagent and alkaline silver nitrate solution.[13]

Results

Renal tubular cell excretion. A difference of 0.18 or more between the logarithms of the control and treatment renal tubular cell counts was considered to be significant and attributable to drug treatment. This figure is the 95 per cent one-sided upper tolerance limit for the differences between control and treatment counts of subjects receiving placebo in a previous study.[17] By this criterion, 2 volunteers receiving phenacetin (Subjects R. L. and M. T.) and one receiving APAP (Subject M. G.) responded with a significant increase in renal tubular cell excretion. There were no significant changes in red blood cell or leukocyte excretion.

Plasma concentrations of phenacetin and APAP. Observations made on the first and fifth treatment days are referred to as the short-term and long-term studies, respectively. Following oral administration, maximum plasma concentrations of phenacetin ranged from 1.3 to 24.8 μg per milliliter and were usually observed within 2 hours. The drug disappeared from the plasma rapidly and only small amounts were present at 8 hours. None could be detected at 24 hours (Table I). There were often marked differences in the individual plasma concentrations in the two studies, and there was considerable intersubject variation in the peak plasma phenacetin concentrations.

The maximum plasma concentrations of free APAP following administration of phenacetin occurred between 2 and 4 hours and ranged from 5.4 to 18.2 μg per milliliter. Easily measurable amounts were present at 8 hours, but none could be detected at 24 hours (Table I).

The plasma half-life of phenacetin varied from 45 to 90 minutes and was relatively constant in the subject irrespective of large differences in plasma concentrations. In most subjects the half-life of free APAP in the plasma was in the range 90 to 120 minutes, and there was a slight tendency toward lower values for both drugs in the long-term compared with the short-term studies (Table I). The plasma half-life and peak concentrations of phenacetin and APAP were not remarkably different in the subjects with a renal tubular cell response (R. L. and M. T.)

The highest plasma concentrations of free APAP following treatment with APAP were observed between 1 and 2 hours (range, 9.9 to 52.3 micrograms per milliliter); the half-life of free APAP in the plasma (75 to 180 minutes) was comparable to that observed after treatment with phenacetin (Table II). The mean plasma concentrations were lower in the long-term study as compared with the short-term study. The plasma concentrations in Subject M. G., who had a renal tubular cell response, were among the lowest observed, although the studies of urinary recovery indicated virtually complete absorption. The plasma half-life in this subject was in the normal range.

Urinary recovery. The percentage of the dose recovered as free and conjugated APAP in the group receiving phenacetin varied from 56 to 98 per cent and appeared to be unrelated to the plasma concentrations of phenacetin (Table I). The mean recovery on the first day of treatment was 74.3 per cent and the higher recovery of 82.3 per cent for the last day probably reflects delayed excretion from previously administered drug. The mean recovery in the subjects receiving APAP was 87 per cent and, similarly, the mean recovery on the last day of treatment was higher than on the first day (Table II). The values for subjects with a renal tubular cell response were well within the normal ranges.

Thin layer chromatography. Several yellow, orange, and violet bands were observed on the unsprayed chromatograms of the glusulase-treated urine extracts from

Table I. *Plasma concentration and half-life of phenacetin and free APAP and urinary recovery*

Subject	Sex	Treatment	Plasma phenacetin concentration (μg/ml.) 1 hr.	2 hr.	4 hr.	6 hr.	8 hr.	24 hr.
L. S.	F	Short-term	1.1	0.8	1.8	0.7	0.2	0
		Long-term	7.7	6.8	1.4	0.4	0.2	0
B. P.	F	Short-term	24.8	15.2	3.4	1.3	0.5	0
		Long-term	6.2	3.3	0.7	0.7	0.3	0
R. L.	F	Short-term	0.5	6.8	1.8	0.7	0.4	0
		Long-term	9.3	4.8	1.7	0.8	0.2	0
M. S.	F	Short-term	2.7	8.1	4.3	0.9	0.5	0
		Long-term	1.5	1.6	3.3	3.4	1.3	0
M. T.	F	Short-term	0.6	3.1	0.6	0.2	0	0
		Long-term	4.3	3.7	0.7	0.2	0	0
E. O.	F	Short-term	12.3	11.6	1.9	0.6	0.1	0
		Long-term	6.5	4.0	0.8	0.4	0.2	0
J. M.	M	Short-term	6.7	5.2	1.9	0.5	0.1	0
		Long-term	1.3	0.5	1.1	0.1	0.2	0
A. W.	M	Short-term	6.8	5.2	0.9	0.3	0	0
		Long-term	0.2	3.8	1.1	0.1	0	0
R. S.	M	Short-term	0.5	19.2	2.6	0.6	0.2	0
		Long-term	22.8	15.8	2.0	0.3	0.2	0
Mean		Short-term	6.2	8.4	2.1	0.6	0.2	0
		Long-term	6.6	4.9	1.4	0.7	0.3	0

subjects given phenacetin (Fig. 1). Although there was considerable variation between individuals, the pigment patterns were not notably different in Subjects M. T. and R. L. Small and variable amounts of 2-hydroxy- and 3-hydroxyphenacetin were tentatively identified on chromatograms sprayed with Pauly reagent. Large amounts of APAP were also observed.

The chromatograms of extracts of glusulase-treated urine from the subjects receiving APAP showed unchanged drug and only traces of pigment. The pattern in Subject M. G. was unremarkable (Fig. 2).

According to Büch and colleagues,[6] the pigments are phenoxazone dyes derived from unstable 2-hydroxyphenetidine (2-hydroxy-4-ethoxyaniline). The latter compound was prepared by acid hydrolysis of authentic 2-hydroxy-phenacetin followed by extraction with methylene dichloride at pH 7.0. The colored extract was evaporated to dryness, dissolved in acetone, and chromatographed. Several colored spots with similar R_f values to some of the urinary pigments were observed.

Untreated and hydrolyzed urine extracts from rats and dogs treated with phenacetin (100 mg. per kilogram) were also chromatographed for comparative purposes. The pigment profiles were similar to those seen with human urine although additional bands were present (Figs. 3 and 4). There was little or no pigment in extracts of unhydrolyzed urine.

Discussion

In the present investigation it was not possible to demonstrate differences in the absorption, excretion, or metabolism of phenacetin or APAP in subjects showing a marked renal tubular cell response com-

as total APAP following oral doses of 1.8 Gm. of phenacetin

Phenacetin half-life (min.)	*Plasma-free APAP concentration (μg/ml.)*						*APAP half-life (min.)*	*% Recovery/ 24 hr. as APAP*
	1 hr.	*2 hr.*	*4 hr.*	*6 hr.*	*8 hr.*	*24 hr.*		
80	2.8	8.1	10.9	8.9	1.5		45	74
70								70
70	6.7	10.3	15.7	7.6	4.0	0	120	66
65	4.8	7.2	7.4	5.6	4.9	0		
70	3.0	12.0	14.4	5.8	2.5	0	92	85
75	8.6	10.7	7.8	3.5	1.4	0	97	94
90	2.8	9.0	5.9	7.5	4.3	0		75
								81
60	4.9	13.2	10.8	5.9	2.5	0	90	56
50	6.0	13.2	7.7	3.4	1.9	0	110	83
50	7.0	18.2	14.2	6.8	3.3	0	120	82
68	8.5	17.5	8.2	5.0	2.1	0	110	76
70	2.9	5.4	4.7	2.5	1.0	0	90	59
	3.4	3.8	5.5	3.3	1.3	0	90	78
50	6.3	15.6	9.9	6.3	3.7	0	170	74
50	2.6	9.9	10.1	5.5	2.6	0	110	81
50	1.6	11.8	11.2	4.8	2.8	0	115	98
45	7.2	10.5	10.0	4.6	0.9	0	70	95
65.6	4.2	11.5	10.9	6.2	2.8	0	105.3	74.3
60.4	5.9	10.4	8.1	4.4	2.2	0	97.8	82.3

pared with those who did not. However, there were considerable variations in the plasma concentrations of phenacetin, both between individuals and in the same individual during the separate studies. Despite this variation, the plasma half-life of phenacetin and free APAP was relatively constant in a given individual. The observed plasma half-life of free APAP agreed with the mean value of 1.95 hours (range, 1.62 to 2.83 hours) reported by Nelson and Morioka.[14] The mean urinary recovery as total APAP following treatment with phenacetin was lower than when APAP was taken. This may have been due either to incomplete absorption of phenacetin or to the formation of metabolites which were not measured as p-aminophenol after hydrolysis of the urine with acid.

Since the effects on renal tubular cell excretion appeared to be unrelated to the plasma concentrations of phenacetin or free APAP, it is possible that metabolites of phenacetin other than APAP are involved. The urine extracts from subjects taking phenacetin contained large amounts of pigment, whereas negligible amounts were present when APAP was taken. These pigments are probably derived from 2-hydroxyphenetidine (2-hydroxy-4-ethoxyaniline). This compound is unstable in alkaline solution and condenses to a yellow dye, 3-amino-7-ethoxyphenoxazone. In man, 8 per cent of a dose of phenacetin was recovered in the urine in 8 hours as the sulfate, 2-sulphonyloxy-4-ethoxyaniline.[6, 7] Pigments similar to those present in human urine were also found in the glusulase-hydrolyzed urine of dogs and rats treated with phenacetin, and, since dogs do not develop renal lesions following chronic administration of large doses of phenacetin,[21] it is unlikely

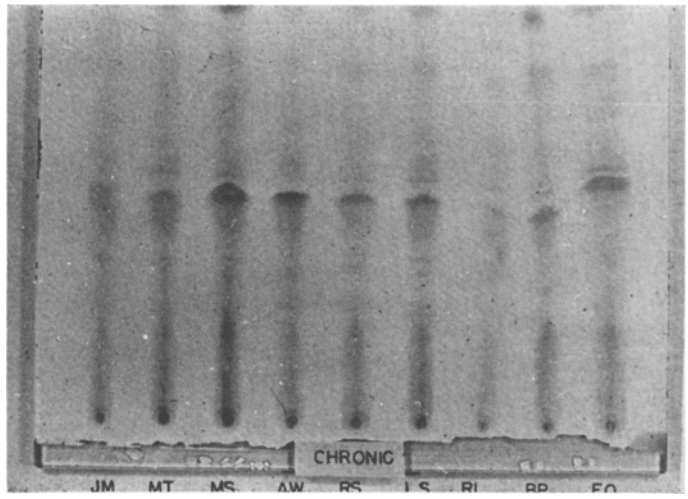

Fig. 1. Unsprayed thin layer chromatograms of extracts of glusulase-hydrolyzed urine from subjects treated with phenacetin for 5 days. Large amounts of orange, yellow, and violet pigments are present.

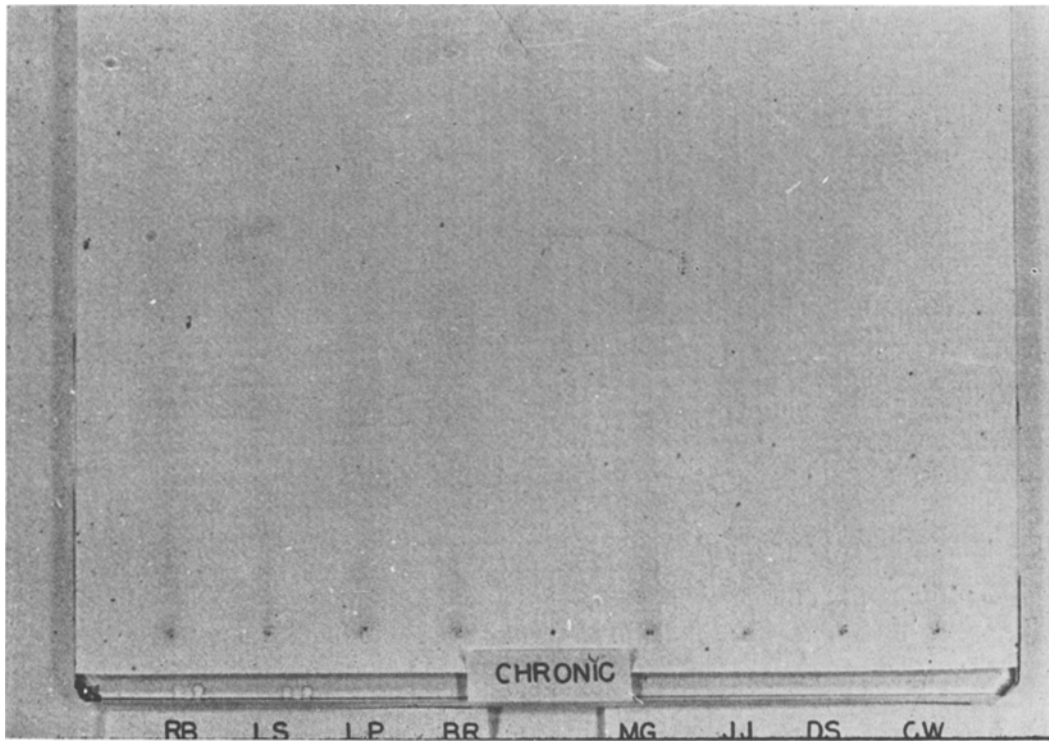

Fig. 2. Thin layer chromatograms of extracts of glusulase-hydrolyzed urine from subjects receiving APAP. Compare with Fig. 1.

Table II. *Plasma concentration and half-life of free APAP and urinary recovery of free and conjugated APAP following oral doses of 1.8 Gm. of APAP*

Subject	Sex	Treatment	Plasma-free APAP concentration (μg/ml.)						Plasma half-life (min.)	% Recovery in urine/ 24 hr.
			1 hr.	2 hr.	4 hr.	6 hr.	8 hr.	24 hr.		
L. S.	M	Short-term	7.6	15.3	7.5	4.1	1.7	0	110	80
		Long-term	12.3	16.8	6.1	3.3	2.4	0	120	92
L. P.	M	Short-term	29.1	13.5	6.1	2.8	1.0	0	90	93
		Long-term	22.1	11.0	3.8	1.3	0.7	0	80	96
M. G.	M	Short-term	5.6	13.3	7.1	2.8	1.5	0	115	93
		Long-term	9.2	9.9	5.5	2.7	1.0	0	115	97
D. S.	M	Short-term	16.9	10.1	3.4	2.1	0.9	0	100	69
		Long-term	20.3	10.1	4.3	1.8	0.4	0	75	73
R. B.	M	Short-term	30.6	19.1	10.3	4.3	2.4	0	120	82
		Long-term	20.9	20.9	9.1	5.0	2.9	0	120	99
J. J.	F	Short-term	44.0	37.5	19.1	10.5	5.5	0	130	49
		Long-term	34.7	26.9	15.5	6.4	3.0	0	115	87
B. R.	F	Short-term	20.2	24.8	15.3	6.8	3.2	0	130	89
		Long-term	11.4	21.5	10.6	3.0	1.8	0	100	86
C. W.	F	Short-term	52.3	38.1	23.4	14.0	9.1	0	180	100
		Long-term	43.3	29.8	14.5	7.6	3.5	0	120	-
Mean		Short-term	25.8	21.5	11.5	5.9	3.2	0	122	82
		Long-term	21.8	18.4	8.7	3.9	2.0	0	106	90

that the metabolites which give rise to these pigments are nephrotoxic.

Other metabolites of phenacetin include p-phenetidine,[3] 2-hydroxy- and 3-hydroxyphenacetin,[7, 13] and possibly N-hydroxyphenacetin.[4, 9] APAP is excreted largely as the glucuronide, ethereal sulfate, and a cysteine conjugate and only 2 to 4 per cent of a dose can be recovered unchanged in the urine.[8, 12] A small amount (less than 1 per cent in man) is deacetylated to primary aromatic amine, but there are marked species differences in this metabolic pathway.[23] Examination of the urines of the subjects taking phenacetin in the present study revealed that less than 0.5 per cent of the dose was present as primary aromatic amine. The amount of amine present in the urine of the 2 subjects with the renal tubular cell response (R. L. and M. T.) was within the range observed for the other subjects.

Little is known of the effects of the minor metabolites of phenacetin on the kidney. Rats, rabbits, and dogs receiving p-phenetidine for 13 to 40 weeks did not develop renal lesions, although there was marked hemolysis.[16, 19] 2-Hydroxyphenacetin had no antipyretic activity in rats and did not cause methemoglobinemia in dogs; no abnormal gross or histological changes were observed in the kidneys of rats receiving 50 mg. per kilogram daily by mouth for 10 days.[9] On the other hand, N-hydroxylation might in this instance be considered a mechanism of toxication rather than detoxication. N-hydroxyphenacetin causes methemoglobinemia in dogs and has antipyretic activity in rats, but no renal lesions were noted in rats receiving 50 mg. per kilogram daily by mouth for 10 days.[9]

Shahidi[20] described the case of a young woman who developed a severe hemolytic

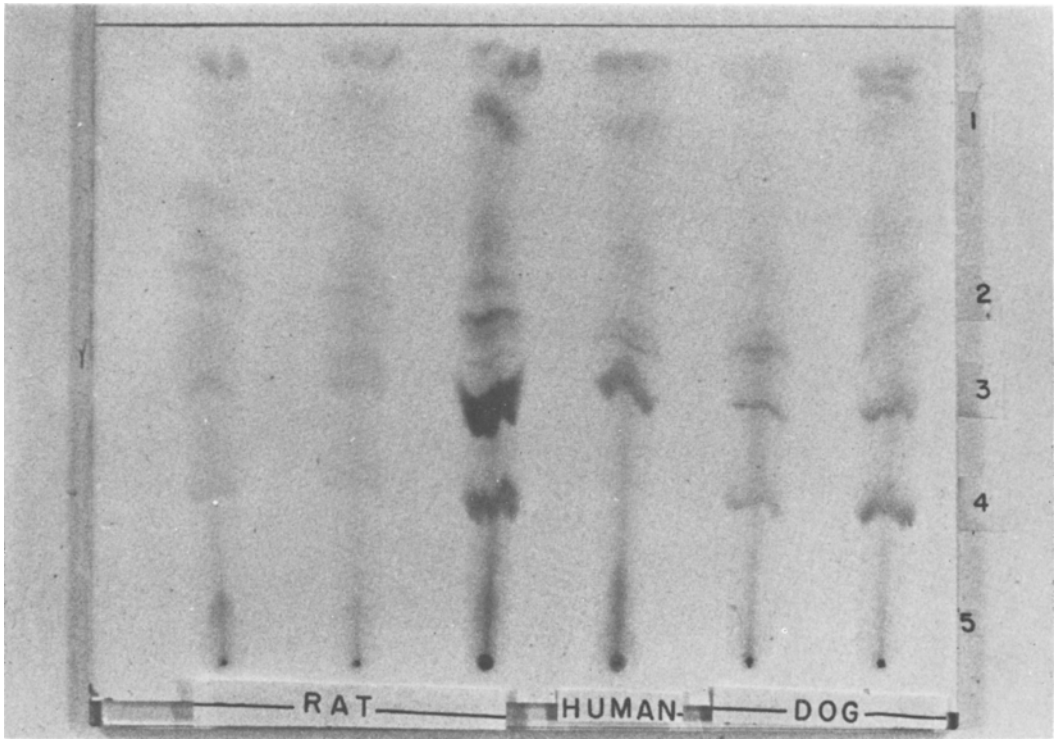

Fig. 3. Thin layer chromatograms of extracts of glusulase-hydrolyzed urine obtained from humans, rats, and dogs treated with phenacetin. Two rats and 2 dogs received 100 mg. phenacetin per kilogram orally. One rat (dark bands on the chromatograms) received 400 mg. of phenacetin per kilogram. The numbers 1 through 5 refer to orange, yellow, violet, violet, and orange spots, respectively.

reaction following ingestion of phenacetin. Large amounts of 2-hydroxyphenacetin and 2-hydroxyphenetidine were present in the urine and only 30 per cent of a dose could be recovered as APAP. Similar abnormalities of phenacetin metabolism were present in the patient's sister, but not in her parents. Dealkylation of 2-hydroxy and 3-hydroxy metabolites could give rise to 4-aminoresorcinol and 4-aminocatechol, respectively. The latter compound is extremely toxic and dogs receiving 10 mg. per kilogram died with hematuria.[15] Furthermore, these metabolites are highly reactive and would probably not be found free in the urine.

The metabolism, and perhaps the toxicity, of phenacetin may be modified by previous or concurrent administration of other drugs. Induction of the drug-metabolizing enzymes of the liver microsomes by pretreatment with phenobarbital or 3-methylcholanthrene in cats and rats results in accelerated metabolism of phenacetin and, under certain conditions, increased formation of methemoglobin.[5, 10, 23] Potentiation of phenacetin toxicity has been observed in man following pretreatment with phenobarbital[20] and chronic administration of drugs may also result in induction of the drug-metabolizing enzymes in man. In the present investigation the mean plasma concentrations of phenacetin and free APAP were lower during the long-term study than on the first day of treatment. Although the differences are not marked, it is possible that the metabolism of phenacetin and APAP in man is stimulated during long-term treatment. Phenacetin is always taken in combination with other drugs such as

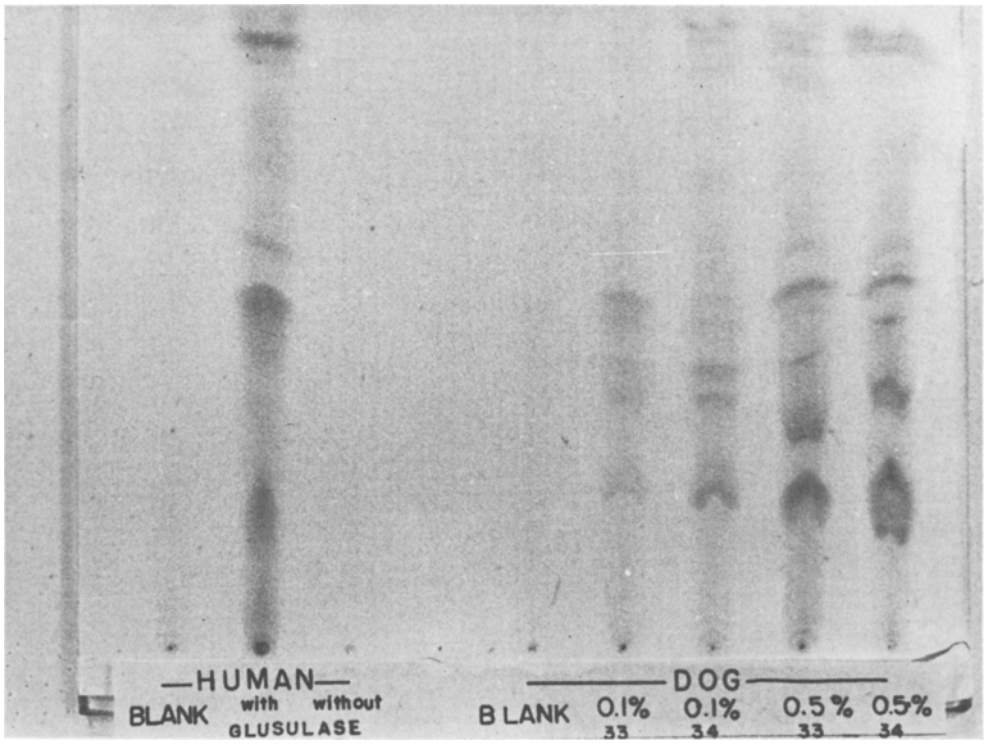

Fig. 4. Thin layer chromatograms of extracts of urine before and after hydrolysis with glusulase in man and dogs receiving phenacetin. Blank urine samples, obtained prior to treatment, were treated with glusulase. One-tenth per cent of a 24 hour urine sample was used for all analyses except for 2 samples of dog urine where 0.5 per cent of the 24 hour urine was used.

salicylates, pyrazolone derivatives, caffeine, or codeine, and patients with analgesic nephritis often take barbiturates regularly. Little is known of the metabolism of phenacetin in man under these conditions.

Summary

The absorption, excretion, and metabolism of phenacetin and N-acetyl-p-aminophenol (APAP) were investigated in conjunction with studies of renal tubular cell excretion in 17 healthy adult volunteers. In each volunteer, plasma concentrations of phenacetin and free APAP and urinary recovery of free and conjugated APAP were measured on the first and fifth days of treatment following an oral dose of 1.8 Gm. of phenacetin or APAP.

Renal tubular cell excretion was markedly increased in 2 of 9 volunteers receiving a total of 13.5 Gm. of phenacetin over 5 days, and in 1 of 8 volunteers receiving the same dose of APAP.

The maximum plasma concentrations of phenacetin varied from 1.3 to 24.8 μg per milliliter, and there were often marked differences in the same individual during the two studies. The half-life of phenacetin in the plasma varied from 45 to 90 minutes and was relatively constant in a given individual despite large differences in plasma concentrations.

There was less variation in the plasma concentrations of free APAP following administration of phenacetin or APAP. With both drugs, the mean plasma concentrations of phenacetin and free APAP were lower on the fifth day of treatment than on the first day. Approximately 78 per cent of the dose of phenacetin and 86 per cent of the APAP was recovered as free and conjugated APAP in the urine in 24 hours.

Many pigmented bands (probably derived from 2-hydroxyphenetidine) were observed on unsprayed thin layer chromatograms of extracts of glusulase-hydrolyzed urine obtained from subjects receiving phenacetin. Similar pigments were present in the hydrolyzed urine of dogs and rats treated with 100 mg. per kilogram of phenacetin. In contrast, negligible amounts of pigment were present in the hydrolyzed urine of the subjects treated with APAP.

Differences in peak plasma concentrations, plasma half-life of phenacetin or free APAP, urinary recovery as free and conjugated APAP, or thin layer chromatographic patterns of urinary metabolites could not be demonstrated between subjects showing a marked renal tubular cell response and those who did not.

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