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Effects of Phenylbutazone Analogues on the Plasma Concentration and Renal Excretion of Salicylate and Its Metabolites in Rabbits[1)]

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Rabbits were intravenously coadministered with salicylate and each of various phenylbutazone analogues (oxyphenbutazone, γ-hydroxyphenylbutazone and ketophenylbutazone). After the drug administration, concentrations of salicylate and its metabolites in the plasma and in the urine were determined by using the GLC method. Salicylate elimination from the plasma was suppressed by oxyphenbutazone and ketophenylbutazone, but was not suppressed by γ-hydroxyphenylbutazone. The renal excretion of salicylate was markedly inhibited by each of the phenylbutazone analogues and the amount of salicylurate excreted was relatively increased, whereas that of salicyl glucuronides was insignificantly affected. Phenylbutazone itself slightly depressed the renal excretion of salicylurate.

Keywords——rabbit; salicylate; phenylbutazone analogues; coadministration; salicylurate; salicyl glucuronides; GLC method; elimination; renal excretion

Salicylate is administered in the form of acetylsalicylic acid (aspirin) which is hydrolyzed rapidly in the body to salicylate.[2)] Aspirin has been widely used not only for antipyretic and analgesic purposes but also as an anti-inflammatory agent, and thus may be suitable for concurrent administration with other anti-inflammatory drugs.[3,4)]

Studies in which aspirin was coadministered with other anti-inflammatory agents such as indomethacin or naproxen have been reported by several investigators,[5–8)] but little work has been done on coadministration of aspirin with phenylbutazone derivatives.

In the previous paper,[1)] the authors demonstrated that when salicylate and phenylbutazone were simultaneously administered to rabbits, the binding ratio of salicylate was decreased but salicylate elimination from the plasma was unexpectedly suppressed because of inhibition of the renal excretion of salicylate by phenylbutazone.

Phenylbutazone is almost completely metabolized in man and animals.[9)] Two metabolites, *viz*, oxyphenbutazone (3,5-pyrazolidinedione-4-butyl-1-(*p*-hydroxyphenyl)-2-phenyl) and γ-hydroxyphenylbutazone (3,5-pyrazolidinedione-4-(3-hydroxybutyl)-1,2-diphenyl) were isolated from the urine of human subjects given phenylbutazone.[10)] Oxyphenbutazone has an anti-inflammatory activity approximately equal to that of the parent drug, while γ-hydroxyphenylbutazone possesses an enhanced uricosuric activity.[11,12)] Ketophenylbutazone (3,5-pyrazolidinedione-4-(3-oxobutyl)-1,2-diphenyl), which has about half the anti-inflammatory activity of phenylbutazone or oxyphenbutazone, is widely used clinically.[13)] The authors therefore attempted to examine whether concurrent administration of salicylate and each of the above phenylbutazone analogues (oxyphenbutazone, γ-hydroxyphenylbutazone and ketophenylbutazone) to rabbits would affect the plasma concentration and urinary excretion of salicylate. In addition, salicylurate and each of the phenylbutazone analogues were intravenously coadministered to other rabbits in order to determine the effects of those analogues on the renal excretion of salicylurate.

Plasma protein binding of salicylate was not examined in this study.

Experimental

Materials——1) Experimental animals: Male albino rabbits (2.8—3.3 kg) fasted for 24 h were used.

2) Drug: Guaranteed reagent sodium salicylate was used. Phenylbutazone (Fujisawa Pharmaceutical Co., Ltd.) and oxyphenbutazone (Sawai Pharmaceutical Co., Ltd.) were recrystallized from ethanol, and ketophenylbutazone (Sawai Pharmaceutical Co., Ltd.) was recrystallized from methanol before use. γ-Hydroxyphenylbutazone was prepared from ketophenylbutazone by using the method of Denss *et al.*[14] Each of the phenylbutazone analogues was administered in the form of the sodium salt, prepared according to the method developed by Tanimura *et al.*[15]

Drug Administration and Sample Collection——Rabbits were intravenously coadministered with 11.6 mg/kg sodium salicylate (equivalent to 10 mg/kg salicylate) and 21.4 mg/kg sodium phenylbutazone analogues (equivalent to 20 mg/kg phenylbutazone analogues) into the ear vein. Furthermore, other rabbits were intravenously administered 10 mg/kg salicylurate with or without 21.4 mg/kg phenylbutazone analogues. Blood samples were taken at the other ear vein for 10 h. Urine samples were collected using a urethral catheter for 24 h at regular intervals after drug administration, and urinary pH was determined with a pH meter (Toa HM-18A) immediately after each urine collection.

Extraction and Quantitative Determination of Drugs——1) Salicylate: Cinnamic acid was used as an internal standard for the analysis of salicylate. Salicylate in plasma and in urine was extracted with ether and 1,2-dichloroethane, respectively, and extracts were trimethylsililated and injected into the gas liquid chromatography (GLC).

2) Salicylurate: Salicylurate in a plasma sample was extracted with ether using mefenamic acid as an internal standard. The extract was methylated with diazomethane and determined. Salicylurate in a urine sample was extracted with ethyl acetate using stearic acid as an internal standard. The extract was trimethylsililated with hexamethyldisilazane and determined.

3) Total Salicylate: Some plasma or urine samples were mixed with concentrated hydrochloric acid and hydrolyzed for 16 h at 100°C. Salicylate in the hydrolyzed sample was extracted and determined in the same way as salicylate in the plasma or urine samples.

Salicylate and salicylurate in all samples were quantitatively determined using the GLC method; the analytical conditions and analytical method were reported in the previous paper.[1]

Results

Rabbits were intravenously coadministered with salicylate and each of the phenylbutazone analogues. The plasma concentrations of salicylate obtained are listed in Table I. The values of the control and after coadministration of salicylate and phenylbutazone are cited from the previous paper.[1] Except for the case of coadministration of salicylate and γ-hydroxyphenylbutazone, salicylate elimination was suppressed by oxyphenbutazone and ketophenylbutazone, and the plasma salicylate levels were significantly higher than those of the

Table I. Plasma Concentration of Salicylic Acid in Rabbits after Intravenous Administration of 11.6 mg/kg Sodium Salicylate

Time (h)	Control[a] (μg/ml)	With PB[a,b] (μg/ml)	With OPB[c] (μg/ml)	With γ-OHPB[d] (μg/ml)	With KPB[e] (μg/ml)
0.5	73.0±17.4	57.2± 6.0	62.5±13.6	60.7±3.5	56.4±8.4
1	57.2± 8.6	64.2± 7.2	52.4± 9.1	45.1±3.2	59.3±7.0
2	35.8± 5.1	59.4±10.3[f]	44.9± 4.3[f]	30.4±4.5	50.7±6.6[f]
3	19.7± 3.7	52.3± 6.0[f]	34.0± 3.9[f]	21.5±6.9	45.2±6.3[f]
4	13.1± 3.0	47.2± 3.6[f]	34.8± 8.1[f]	13.5±6.5	41.8±9.7[f]
6	9.4± 2.5	37.3± 4.6[f]	27.0± 8.0[f]	14.2±0.7	34.5±7.1[f]
8	4.6± 2.4	34.4± 1.9[f]	22.2± 4.6[f]	7.4±5.2	28.3±7.0[f]
10	2.9± 1.6	23.8	18.6± 6.3[f]	5.4±2.1	21.2±4.7[f]

a) These data are cited from the previous paper.[1]
b) Simultaneously administered with sodium phenylbutazone (21.4 mg/kg, *i.v.*).
c) Simultaneously administered with sodium oxyphenbutazone (21.4 mg/kg, *i.v.*).
d) Simultaneously administered with sodium γ-hydroxyphenylbutazone (21.4 mg/kg, *i.v.*).
e) Simultaneously administered with sodium ketophenylbutazone (21.4 mg/kg, *i.v.*).
f) Significantly different from the control ($p<0.05$).
Each value represents the mean±S.D. from 3—4 experiments.

control in the periods later than 2 h after administration. In the case of coadministration of salicylate and γ-hydroxyphenylbutazone, plasma salicylate levels were not significantly different from those of the control.

The metabolites of salicylate in the plasma were determined in each experiment, but only salicylurate was detected in a negligible quantity.

Therefore, in order to find the reason for the suppression of salicylate elimination from the plasma, the urinary excretion of salicylate was determined. Salicylate is excreted in the forms of unchanged salicylate, glycine conjugate (salicylurate) and two types of glucuronic acid conjugates (acyl glucuronide and phenolic glucuronide).[2] The determinations of salicylate, salicylurate and total salicylate were carried out directly by application of colorimetric estimation[16] to the GLC method in the same way as described in the previous paper.[1] The determination of glucuronic acid conjugates was not done directly, but the difference between the amount of total salicylate and the sum of amounts of salicylate and salicylurate was taken as the quantity of glucuronic acid conjugates.

Data on the urinary excretion of salicylate obtained after the administration of sodium salicylate alone are represented in Fig. 1 (control); these results are cited from the previous paper.[1] Cumulative excretion of unchanged salicylate during 24 h was about 70% of the dose, and those of salicylurate and salicyl glucuronides were 6.5% and 13.8%, respectively.

The urinary excretion of salicylate when sodium salicylate and sodium oxyphenbutazone were coadministered is shown in Fig. 2. Cumulative excretion of unchanged salicylate during 24 h amounted to about 25% of the dose, and was significantly decreased as compared with that in the control, which was higher than that obtained upon coadministration of sodium salicylate and sodium phenylbutazone, reported in the previous paper.[1] Cumulative excretion of salicylurate was increased to 23.2% of the dose, but the difference from the control was

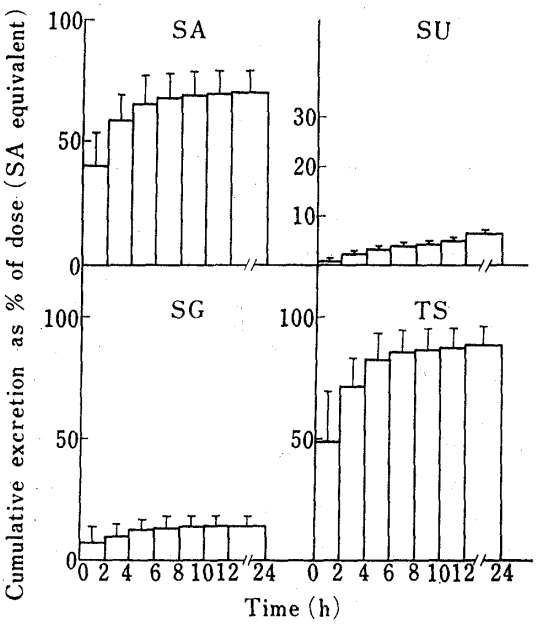

Fig. 1. Urinary Excretion of Salicylic Acid and Its Metabolites

Rabbits were intravenously administered sodium salicylate (11.6 mg/kg) alone.
SA: salicylate; SU: salicyluric acid; SG: salicyl glucuronides=TS−(SA+SU); TS: total salicylate.

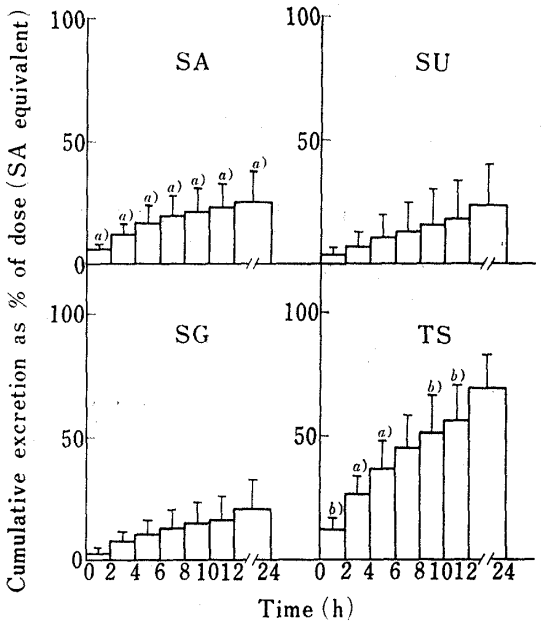

Fig. 2. Effects of Oxyphenbutazone on the Urinary Excretion of Salicylic Acid and Its Metabolites

Sodium oxyphenbutazone (21.4 mg/kg, *i.v.*) was simultaneously administered with sodium salicylate (11.6 mg/kg, *i.v.*). SA: salicylate; SU: salicyluric acid; SG: salicyl glucuronides=TS−(SA+SG); TS: total salicylate. Statistically significant differences against the control (see Fig. 1) are indicated by the following marks; *a*) $p<0.01$, *b*) $p<0.05$.

not significant. Total salicylate excreted was significantly decreased during 12 h but was insignificantly different in 24 h after drug administration. The excreted quantity of salicyl glucuronides calculated according to the previously described method during 24 h was increased to 20.7%, which is not significantly different from the control statistically.

The urinary excretion of salicylate after coadministration of sodium salicylate and sodium γ-hydroxyphenylbutazone is represented in Fig. 3. Cumulative excretion of unchanged salicylate during 24 h was significantly decreased to 21.4% of the dose as compared with the control, which gave a value as high as that in the case of coadministration of sodium salicylate and sodium oxyphenbutazone. In contrast, cumulative excretion of salicylurate was markedly increased to 46.3%, and that of salicyl glucuronides was insignificantly different from that of the control. Cumulative excretion of total salicylate was not significantly different from that of the control throughout the entire experimental period.

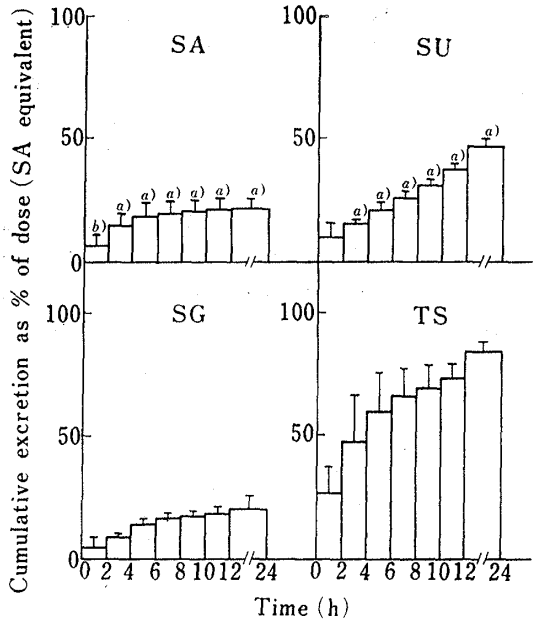

Fig. 3. Effect of γ-Hydroxyphenylbutazone on the Urinary Excretion of Salicylic Acid and Its Metabolites

Sodium γ-hydroxyphenylbutazone (21.4 mg/kg, *i.v.*) was simultaneously administered with sodium salicylate (11.6 mg/kg, *i.v.*). SA: salicylate, SU salicyluric acid, SG: salicyl glucuronides=TS−(SA+SU), TS: total salicylate. Statistically significant differences against the control (see Fig. 1) are indicated by the following marks; *a*) $p<0.01$, *b*) $p<0.05$.

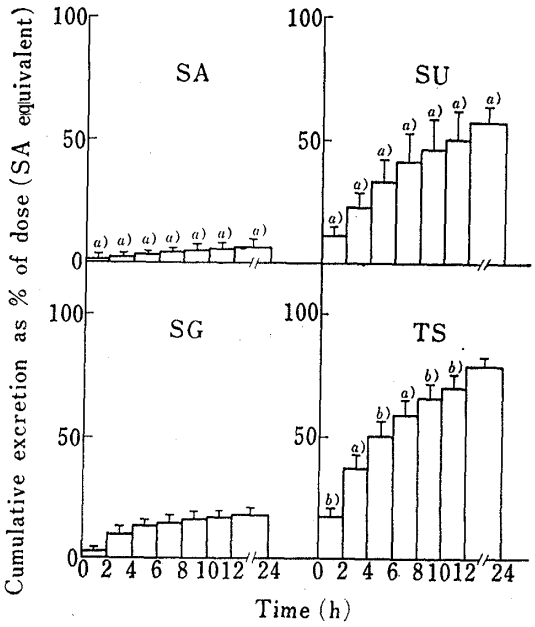

Fig. 4. Effect of Ketophenylbutazone on the Urinary Excretion of Salicylic Acid and Its Metabolites

Sodium ketophenylbutazone (21.4 mg/kg, *i.v.*) was simultaneously administered with sodium salicylate (11.6 mg/kg, *i.v.*). SA: salicylate, SU: salicyluric acid, SG: salicyl glucuronides=TS−(SA+SU), TS: total salicylate. Statistically significant differences against the control (see Fig. 1) are indicated by the following marks; *a*) $p<0.01$, *b*) $p<0.05$.

The urinary excretion of salicylate after coadministration of sodium salicylate and sodium ketophenylbutazone is represented in Fig. 4. The results are different from the cases in which sodium salicylate was simultaneously administered with sodium oxyphenbutazone or sodium γ-hydroxyphenylbutazone. That is, cumulative excretion of unchanged salicylate during 24 h was markedly decreased to about 6.1%, which was as little as that in the coadministration of sodium salicylate and sodium phenylbutazone (see the previous paper[1]). In contrast, that of salicylurate was markedly increased to 57.1%, but that of salicyl glucuronides was about 17.6%, almost the same as the control. Cumulative excretion of total salicylate was significantly decreased during 12 h after the administration, but no significant difference was found in 24 h as compared with the control.

From these findings, it is clear that each of the phenylbutazone analogues inhibits the renal excretion of salicylate to some extent, but it is not obvious that they inhibit the renal

excretion of salicylurate because the data include the effect of metabolism of salicylate. Thus, in order to find out whether these analogues inhibit the renal excretion of salicylurate, a group of rabbits was intravenously given salicylurate with or without each of the phenylbutazone analogues. The results are represented in Figs. 5 and 6. Only phenylbutazone inhibited the renal excretion of salicylurate, but the inhibition of the renal excretion of salicylurate

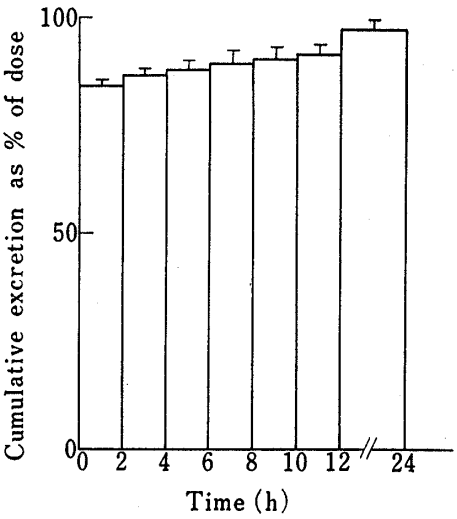

Fig. 5. Urinary Excretion of Salicyluric Acid in Rabbits

Rabbits were intravenously administered salicylurate (10 mg/kg) alone.

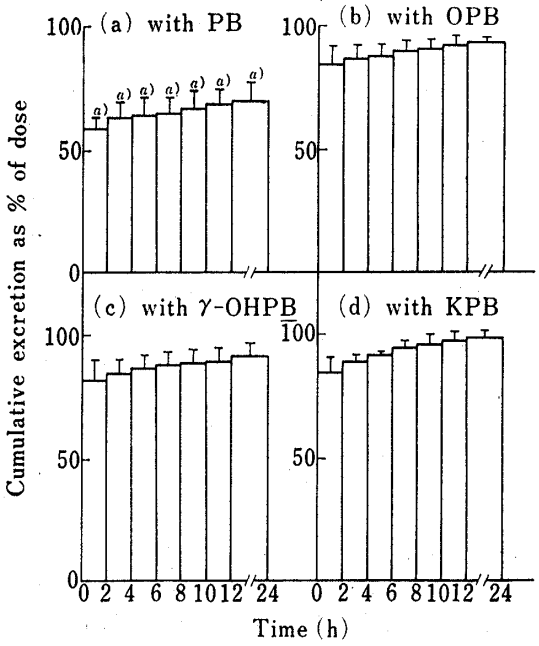

Fig. 6. Effects of Phenylbutazone Analogues on the Urinary Excretion of Salicyluric Acid

(a): salicylurate (10 mg/kg, *i.v.*)+sodium phenylbutazone (21.4 mg/kg, *i.v.*).
(b): salicylurate (10 mg/kg, *i.v.*)+sodium oxyphenbutazone (21.4 mg/kg, *i.v.*).
(c): salicylurate (10 mg/kg, *i.v.*)+sodium γ-hydroxyphenylbutazone (21.4 mg/kg, *i.v.*).
(d): salicylurate (10 mg/kg, *i.v.*)+sodium ketophenylbutazone (21.4 mg/kg, *i.v.*).
Statistically significant differences against the control (see Fig. 5) are indicated by the following mark; *a*) $p<0.05$.

TABLE II. Urinary pH in Rabbits after Intravenous Administration of 11.6 mg/kg Sodium Salicylate

Time (h)	Control[a]	With PB[a,b]	With OPB[c]	With γ-OHPB[d]	With KPB[e]
0	7.04±0.04	7.15±0.39	7.26±0.94	7.08±1.20	7.16±0.65
2	7.16±0.47	6.93±0.13	6.80±0.89	6.95±0.66	6.57±0.82
4	6.69±0.58	6.47±0.54	6.56±0.92	6.70±0.86	6.06±0.38
6	6.67±0.21	6.34±0.51	6.46±1.19	6.25±0.97	5.84±0.20[f]
8	6.47±0.30	6.21±0.50	6.32±0.94	6.03±0.87	5.60±0.27[f]
10	6.13±0.61	6.31±0.14	6.14±0.87	5.74±0.86	5.58±0.21
12	5.41±0.48	5.99±0.40	6.06±0.69	5.55±0.69	5.45±0.14
24	4.94±0.21	5.32±0.30	5.56±0.83	5.01±0.16	4.87±0.05

a) These data are cited from the previous paper.[1]
b) Simultaneously administered with sodium phenylbutazone (21.4 mg/kg, *i.v.*).
c) Simultaneously administered with sodium oxyphenbutazone (21.4 mg/kg, *i.v.*).
d) Simultaneously administered with sodium γ-hydroxyphenylbutazone (21.4 mg/kg, *i.v.*).
e) Simultaneously administered with sodium ketophenylbutazone (21.4 mg/kg, *i.v.*).
f) Significantly different from the control ($p<0.05$).

by phenylbutazone was weaker than the effect on that of salicylate, and the other analogues did not inhibit the renal excretion of salicylurate.

Furthermore, in these experiments, salicylurate elimination from the plasma was remarkably rapid because salicylurate is more water-soluble than salicylate, and salicylurate was no longer detectable in the plasma later than 4—6 h after administration.

Table III. Urinary pH in Rabbits after Intravenous Administration of 10 mg/kg Salicyluric Acid

Time (h)	Control	With PB[a]	With OPB[b]	With γ-OHPB[c]	With KPB[d]
0	6.94±1.42	7.02±0.69	7.42±0.84	7.39±0.21	6.98±0.87
2	6.54±1.12	6.65±1.23	6.73±0.90	7.98±1.31	6.99±0.47
4	6.55±0.81	6.34±0.44	6.68±1.03	7.95±1.16	6.53±0.46
6	6.38±0.63	6.14±0.46	6.59±1.08	6.26±1.03	6.48±0.30
8	6.34±0.48	5.71±0.52	6.08±1.25	6.55±0.71	6.19±0.42
10	6.00±0.57	5.41±0.30[e]	6.29±1.10	5.97±0.48	5.72±0.42
12	5.77±0.50	5.25±0.56	5.72±0.40	5.51±0.07	5.75±0.36
24	5.20±0.37	5.05±0.21	5.36±0.44	4.98±0.10	5.21±0.45

a) Simultaneously administered with sodium phenylbutazone (21.4 mg/kg, *i.v.*).
b) Simultaneously administered with sodium oxyphenbutazone (21.4 mg/kg, *i.v.*).
c) Simultaneously administered with sodium γ-hydroxyphenylbutazone (21.4 mg/kg, *i.v.*).
d) Simultaneously administered with sodium ketophenylbutazone (21.4 mg/kg, *i.v.*).
e) Significantly different from the control ($p<0.05$).

The urinary excretions of salicylate and salicylurate are remarkably affected by the pH of urine.[17–19] Urinary pH after the drug administration was measured (Tables II and III). The pH gradually decreased with time in each experiment, but there were no significant differences among the data. These findings suggest that changes of the eliminated fraction of salicylate and its metabolites are not due to the change of urinary pH after drug administration.

Discussion

Smith *et al.* and Levy have pointed out that the magnitude of salicylate blood levels at various times after the administration of salicylate may be altered depending on the dose, dissolution rate, absorption rate, gastric emptying time, permeability characteristics of the gastrointestinal membranes, plasma protein binding, and rates of metabolic conversion and renal excretion.[20] Among these factors, plasma protein binding and alterations of metabolic conversion and renal excretion are factors directly influencing the salicylate blood level. Protein binding of salicylate was not examined in this experiment, but salicylate is known to be displaced by phenylbutazone from its binding sites.[1,21–23] Two metabolites of phenylbutazone, oxyphenbutazone and γ-hydroxyphenylbutazone, are also known to bind to plasma protein.[24] Therefore, it can be considered that salicylate is probably displaced from its binding sites when it is coadministered with oxyphenbutazone or γ-hydroxyphenylbutazone. However, salicylate elimination was not enhanced; indeed, it was observed that salicylate elimination from the plasma was markedly suppressed by phenylbutazone, oxyphenbutazone and ketophenylbutazone.

Limited capacity for salicylurate formation from salicylate has been reported.[25,26] Furthermore, Levy *et al.* recently proposed the existence of a limited capacity for salicyl phenolic glucuronide formation from salicylate.[27] Therefore, it is considered that salicylate accumulation in tissues or the kidneys might occur as a results of saturation owing to the suppression of salicylate elimination from plasma by phenylbutazone and its analogues.

Since salicylurate is more water-soluble than salicylate, the excretion of the former is more rapid than that of the latter.[28] The results obtained here are in good agreement with

this result (see Figs. 1 and 5). Therefore, it can be considered that the formation of salicylurate from salicylate is the rate-limiting step in the excretion of salicylurate after the administration of salicylate; the same suggestion was made by Levy *et al.*[29]

The findings obtained in this experiment show that phenylbutazone and ketophenylbutazone have higher inhibitory potencies for the renal excretion of salicylate than oxyphenbutazone or γ-hydroxyphenylbutazone. Despopoulos *et al.* studied the effects of phenylbutazone derivatives on the renal excretion of *p*-aminohippurate in detail, that is, an increase of alkyl chain length at the 4-position of phenylbutazone derivatives seemed to be associated with increased inhibitory potency for the renal transport of *p*-aminohippurate. In contrast, hydroxylation appreciably reduced the activity, whether the substitution was in the phenyl ring or in the side chain at the 4-position.[30] As judged from the evidence obtained here and their conclusions, hydroxylation in the phenyl ring or in the side chain at the 4-position of phenylbutazone derivatives seems to be associated with decreased inhibitory potency for the renal excretion of drugs. In addition, King has reported that phenylbutazone inhibits the renal excretion of *p*-aminohippurate.[31] In this experiment, however, phenylbutazone analogues inhibited the renal excretion of salicylate but did not inhibit that of salicylurate. Salicylurate might not be secreted by *p*-amincehippuric acid (PAH) mechanism in renal tubuli, or salicylate and salicylurate might be secreted by different mechanisms.

Salicylate is mainly metabolized by conjugation with glycine and glucuronic acid, while phenylbutazone is mainly hydroxylated. Dieterle *et al.*, however, have recently demonstrated that phenylbutazone and its analogues are conjugated with glucuronic acid.[32] In this experiment, the renal excretion of salicylate was markedly inhibited by each of the phenylbutazone analogues tested, and the excretion amount of salicylurate was relatively increased, while that of salicyl glucuronides was insignificantly affected. Phenylbutazone and its analogues may compete with salicylate for glucuronic acid conjugation.

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