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Tolbutamide–Sulfaphenazole Interaction in Rabbits

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The kinetics of tolbutamide–sulfaphenazole interactions were studied in rabbits. Analysis of blood level data showed that sulfaphenazole displaces tolbutamide from serum protein to increase unbound tolbutamide, which results in enhancement of the elimination of tolbutamide from the blood. This finding of enhanced elimination of tolbutamide from blood is quite different from the results of earlier researchers, who found that, in man, a marked prolongation of tolbutamide blood half-life occurred without exception. Inhibitory effects of sulfaphenazole on urinary excretion of the metabolites of tolbutamide were also observed in this study.

KEY WORDS: tolbutamide–sulfaphenazole interaction; protein binding displacement; species difference.

INTRODUCTION

Incidents of severe hypoglycemia in diabetic patients have been reported when tolbutamide (TB) and sulfaphenazole (SP) were administered together. Two possible mechanisms by which a hypoglycemic effect of TB is potentiated by SP are proposed: (a) SP inhibits TB oxidation to prolong the maintenance of TB in blood, and (b) SP displaces TB from serum protein to increase unbound TB. The former mechanism has been demonstrated not only by *in vitro* microsomal experiments on liver in man (1) and rabbit (2) but also by *in vivo* experiments in man (3–5). The latter mechanism, however, has been demonstrated only by *in vitro* experiments (3,6), and its contribution appears to be of less consequence than the former. It has been suggested that SP-induced hypoglycemia is caused by an accumulation of TB and that a contributory factor may be that a much higher

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than normal proportion of this increased amount of serum TB is not protein bound (7).

In this study, rabbits were used to investigate in further detail the mechanisms involved in the interaction of TB and SP. The present study is based on earlier pharmacokinetic research conducted in our laboratory (8) which explored the relevance of the protein binding of TB.

EXPERIMENTAL

Animal Experiments

Male albino rabbits weighing 2.2–3.4 kg were used. They were starved overnight before and during the experiment. The interaction of TB and SP was examined by the following two experimental protocols.

Bolus Experiment

For the bolus experiment, a 150 mg/kg dose of TB was administered via the ear vein and the same dose of SP was given similarly 5 hr later. In addition, SP was given orally. TB was dissolved in an equimolar NaOH solution (20 ml). SP solution was prepared from the commercially available injection solution (10%, w/v, Dainippon Pharmaceutical Co., Osaka, Japan).

Infusion Experiment

In the infusion experiment, TB was infused for 10 hr intraperitoneally at a constant rate (50 mg/hr) using a KN infusion pump (Natsume Seisakusho, Tokyo, Japan). Adjustment of the infusion rate on a body weight basis was difficult because of the pumping system. The mean rate, expressed in mg/kg/hr, was 18.5 since the mean body weight of the seven rabbits used for this experiment was 2.7 kg. A 150 mg/kg dose of SP was given in the ear vein 8 hr after the initiation of TB infusion. TB solution for the infusion was prepared by dissolving 600 mg of TB in an equimolar NaOH solution (100 ml), which was then infused at a rate of 8.3 ml/hr. SP solution was prepared as in the bolus experiment.

Blood and Urine Collection

In both experiments, blood specimens were taken with a syringe containing 3.8% sodium citrate solution. Blood collections were made hourly or every half hour. Urine collections were made hourly through a catheter inserted into the bladder. Five to ten milliliters of water was used to rinse the bladder at every collection and the washings were combined for the analysis.

Materials

TB and SP injection solution was of pharmaceutical grade. All other chemicals were of reagent grade.

Analytical Methods

TB in blood was determined by selective extraction; this had been established in a previous study (9) and by specific coloration with 2,4-dinitrofluorobenzene (DNFB). This coloration made it possible to determine TB alone, even when SP or its metabolites were present concomitantly.

In most cases, whole blood levels of TB were measured instead of plasma levels, because it was experimentally difficult to collect sufficient volumes of blood so frequently from a single rabbit and preliminary study of some blood specimens from rabbits dosed with TB revealed that the plasma levels were only slightly higher than whole blood levels. It should also be noted that TB levels measured in this study correspond to the sum of protein-bound and free species (8).

TB in Blood and Plasma

For the determination of TB in blood and plasma, 0.5 ml of citrated blood or plasma was mixed with 3.0 ml of purified water, and then 1.0 ml of *p*H 5.0 phosphate buffer (0.5 M) and 3.0 g of NaCl were added. The mixture was shaken with 25.0 ml of heptane–chloroform (8:2) for 20 min. After centrifugation, 20.0 ml of the organic solvent phase was shaken with 5.0 ml of 0.1 N NaOH solution for 20 min. Four milliliters of the alkaline phase was added with 0.5 ml of 3 N HCl and again extracted with 6.0 ml of isoamyl acetate for 20 min. Next, 5.0 ml of the solvent phase was reacted with 0.5 ml of 0.1% DNFB (isoamyl acetate solution) at 145°C for 7 min and allowed to stand at room temperature for 1 hr. The absorbance was measured at 350 nm, using the appropriately extracted blank blood for zero setting. It was confirmed that the determination of TB by this method was not affected by the presence of 5 times more SP than TB.

HTB and CTB in Urine

The TB metabolites hydroxymethyltolbutamide (HTB) and carboxytolbutamide (CTB) were determined in urine contaminated with SP and its metabolites by the methods reported previously (9) without any modification.

HTB and CTB in Plasma

HTB and CTB were determined in plasma by quantitative thin-layer chromatography. In this procedure, 2.0 ml of plasma was combined with

2.0 ml of purified water and 0.8 ml of 1 N HCl, and then this mixture was shaken with 20.0 ml of isoamyl acetate for 20 min.[2] After centrifugation, 16.0 ml of the solvent phase was evaporated to dryness under reduced pressure. The residue was dissolved in about 0.5 ml of acetone, and the total amount was applied to form a narrow band on a 20- by 20-cm silica gel HF_{254} plate (E. Merck, Darmstadt, West Germany). After developing with the solvent system ethyl acetate–methanol–benzene (6 : 1 : 4), the spots were detected under ultraviolet light. The R_f values of authentic TB, HTB, and CTB were around 0.7, 0.35, and 0.05, respectively. The entire silica gel within each zone corresponding to HTB and CTB was scraped off and extracted with 6.0 ml of 0.1 N NaOH for 20 min. After centrifuging, 5.0 ml of the supernatant phase was acidified by adding 0.5 ml of 3 N HCl and then shaken with 6.0 ml of isoamyl acetate. Next, 5.0 ml of the organic solvent phase was reacted with 0.5 ml of 0.1% DNFB solution at 145°C for 7 min and allowed to stand at room temperature for 1 hr. The absorbance was measured at 350 nm against the plasma blank run through the above procedure, which gave almost same absorbance as that of the reagent blank. For preparing the calibration curves, 2.0 ml of citrated blank plasma was added with 2.0 ml of the standard solution of HTB or CTB (2.5, 5.0, 7.5, and 10.0 mg %) and 0.8 ml of 1 N HCl; then the mixture was treated as above. The absorbances were found to be proportionate to the amounts of the metabolites added. Those for 10.0 mg % standard solutions of HTB and CTB were 0.24 and 0.38, respectively.

Blood Sugar

Blood sugar was measured by the method of Somogyi (10) using 0.1 ml of blood.

RESULTS AND DISCUSSION

Effect of SP on TB Elimination from Blood

Blood levels of TB found in the bolus experiment are listed in Table I, and the mean values are plotted on a logarithmic scale against time in Fig. 1. Sudden drops of TB blood levels were observed at 1 hr after SP administration. The mean half-lives of TB before and after SP were 4.5 hr (SD = 0.54) and 4.1 hr (SD = 0.85), but the difference was not statistically significant. These findings are quite different from those observed earlier in man (3–5),

[2]It has been confirmed that TB, HTB, and CTB are completely extracted into the solvent under these conditions (9).

Table I. Mean Blood Levels After Intravenous Injection of Tolbutamide with Sulfaphenazole Given by Intravenous Bolus or Oral Route

Time (hr)	Blood level (mg %, mean ± SD)	
	Intravenous group[a]	Oral group[b]
1	39.78 ± 3.70	37.41 ± 1.94
2	31.33 ± 5.21	33.48 ± 0.86
3	27.06 ± 4.13	28.68 ± 3.20
4	23.69 ± 3.59	24.20 ± 3.03
5[c]	20.42 ± 3.78	20.63 ± 1.85
6	14.80 ± 2.86	14.66 ± 1.75
7	12.68 ± 2.53	12.08 ± 1.91
8	10.10 ± 3.00	9.97 ± 1.42
9	9.18 ± 2.07	8.02 ± 1.09
10	7.33 ± 1.81	6.58 ± 1.75

[a]Values for 11 rabbits.
[b]Values for two rabbits.
[c]Sulfaphenazole was given immediately after the collection of the 5-hr blood sample.

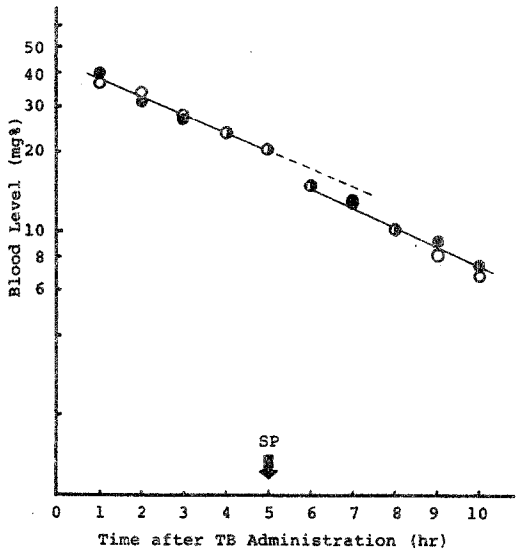

Fig. 1. Blood levels of TB after an intravenous bolus of TB (150 mg/kg) and a subsequent dose of SP (150 mg/kg) by an intravenous bolus (●) or an oral route (○). The half-filled circles indicate that the data for both are practically the same.

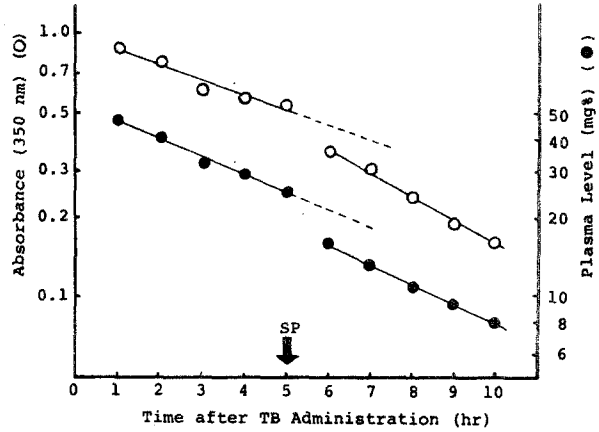

Fig. 2. Comparison between plasma levels of TB as measured in the same specimens by the method of Spingler (○) and that of the present authors (●). TB (150 mg/kg) and SP (150 mg/kg) were given by an intravenous bolus. Where Spingler's method was used, the levels are expressed as absorbance values.

in whom, without exception, SP caused a marked prolongation of TB serum (3,4) and plasma (5) half-lives.

In this context, plasma levels of TB were also measured in two rabbits. The data are plotted in Fig. 2. Comparison of these blood and plasma data indicates that the overall elimination patterns are apparently same, even though the plasma levels are consistently a little higher than the corresponding blood levels, as suggested in the Experimental section. This was further confirmed by comparing the volume of distribution of TB computed from the dose (150 mg/kg) and the extrapolated zero-hour blood or plasma concentration, which was approximately 340 ml/kg relative to the blood data and approximately 300 ml/kg relative to the plasma data. In supplementary experiments in which SP was given orally to examine the influence of route of administration, the same tendency was observed, indicating that the route of SP administration was not essentially responsible for the results.

It was first suspected that the difference between the results of the present study and those of previous studies was due to the analytical methods that were employed for TB. In the previous studies (3,4), Spingler's method (11) was used, which possibly included HTB and CTB in addition to TB in the estimation. The method used in this study determined the presence of TB selectively, even though TB was contaminated with its metabolites and SP-related species. Therefore, comparative determinations by the methods of Spingler and the present authors were done on the same

Table II. Mean Blood Levels of Tolbutamide Obtained from the Infusion Experiment

Time (hr)	Blood level[a] (mg%, mean ± SD)
1	6.70 ± 1.14
2	12.61 ± 1.49
3	16.73 ± 1.72
4	19.65 ± 1.89
5	21.32 ± 1.42
6	22.70 ± 2.66
7	23.31 ± 2.64
7.5	24.09 ± 3.01
8[b]	23.91 ± 2.99
8.5	20.18[c] ± 2.58
9	21.13 ± 2.18
9.5	21.46 ± 2.64
10	21.96 ± 2.67

[a]Values for seven rabbits.
[b]Sulfaphenazole was given intravenously immediately after collection of the 8-hr blood sample.
[c]Significantly lower than the level at 8 hr ($p < 0.05$).

plasma samples from two rabbits. The results are shown in Fig. 2; it was found that overall patterns were essentially the same for both methods, suggesting that the difference cannot be explained in terms of analytical methodology.

Evidence that more clearly implicates SP in the sudden decrease of TB blood levels was found in the infusion experiment. TB blood levels characteristically become constant at some plateau level if the infusion is continued in this kind of experiment. TB blood levels of seven rabbits at time intervals of 1–10 hr are shown in Table II.[3] The mean TB blood levels are plotted against time in Fig. 3. TB blood levels continued to increase and reached a plateau level of almost 25 mg % about 8 hr after the infusion was begun. At that time, SP was given by an intravenous bolus. A sudden drop in

[3]The volume of distribution (V_d) of TB was also estimated in this case by substituting a set of typical experimental data into the following equation:

$$C_b = \frac{k_0}{V_d \cdot K}(1 - e^{-Kt})$$

where C_b is the blood level at time t (24.1 mg % at 7.5 hr), and k_0 and K represent the infusion rate (18.5 mg/kg/hr) and the elimination rate constant (0.154 hr^{-1} from the bolus experiment), respectively. The estimated V_d was found to be approximately 330 ml/kg, and this is comparable to the value found in the bolus experiment (340 ml/kg).

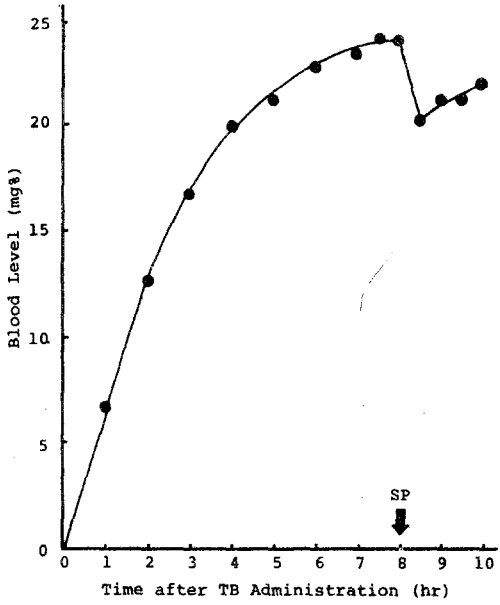

Fig. 3. Blood levels of TB after a constant-rate infusion of TB (50 mg/hr) and a subsequent intravenous bolus of SP (150 mg/kg).

the TB blood level and a subsequent restoration were clearly evident. The mean TB blood level at 0.5 hr after SP administration was significantly lower than that just before SP administration ($p < 0.05$).

When SP is administered as a bolus injection, the TB blood levels and plasma levels drop as indicated in Figs. 1 and 2. This abrupt drop cannot be explained as being due to the inhibition of TB metabolic oxidation since this should decrease the slope of the elimination curve. The mean half-lives before and after SP administration computed from these data did not differ significantly, as suggested above. Since the levels are abruptly shifted downward after the administration of SP, but still have almost the same mean slope, the resultant data appear to be compatible with the interpretation that SP displaces TB from the plasma protein. The SP-altered elimination of TB should be changing corresponding to the concentration of SP which is decreasing with time. This time course of the SP effect could not be clearly judged from the data obtained from the bolus experiment. However, the data from the infusion experiment, depicted in Fig. 3, indicate an apparently time-dependent reversion to the original elimination characteristics of TB. This distinctive aspect of the interaction of TB and SP should

be further pursued by examining the details of SP disposition in rabbits, and these are currently under investigation in our laboratory.

Effect of SP on HTB and CTB Excretion in Urine

The urinary excretion rates of HTB and CTB were investigated concurrently with the blood level studies discussed above. Because the mechanism above suggested that SP enhances the oxidation of TB, an increase in excretion rates of TB metabolites was expected. However, as shown in Tables III and IV and Figs. 4 and 5, a decrease in excretion rates was found, with the sudden falls immediately after SP administration being inconsistent with the enhanced elimination of TB from blood. These excretion patterns affected by SP might be reconciled if it is considered that the inhibitory mechanisms may intervene somewhere in the processes following the formation of HTB from TB.

Effect of SP on Disposition of HTB and CTB

In order to better understand the implications of the facts that SP enhances TB elimination from blood and also blocks subsequent steps leading to the excretion of HTB and CTB in urine, the plasma levels of HTB and CTB were examined using 8-, 8.5-, and 10-hr plasma samples from four rabbits used in the infusion experiment described earlier. The results, summarized in Table V and Fig. 6, demonstrate that CTB plasma levels increase significantly ($p < 0.05$) upon SP administration, while HTB plasma

Table III. Mean Urinary Excretion Rates of Metabolites of Tolbutamide After Intravenous Injection

	Urinary excretion rate[a] (mg/hr, mean ± SD)	
Time (hr)	Hydroxymethyltolbutamide	Carboxytolbutamide
1	11.45 ± 7.33	32.15 ± 13.18
2	16.56 ± 5.01	48.84 ± 4.08
3	13.98 ± 3.61	37.12 ± 7.54
4	11.74 ± 2.61	32.03 ± 8.12
5[b]	10.70 ± 2.09	23.89 ± 5.85
6	5.54 ± 1.73	10.96 ± 3.03
7	6.61 ± 1.33	14.50 ± 4.59
8	6.89 ± 1.34	13.55 ± 5.11
9	5.69 ± 1.13	7.90 ± 1.46
10	3.63 ± 1.35	7.83 ± 2.47

[a]Values for seven rabbits, expressed as mg/hr of tolbutamide.
[b]Sulfaphenazole was given intravenously immediately after collection of the 5-hr urine sample.

Table IV. Mean Urinary Excretion Rates of Metabolites of Tolbutamide Obtained from the Infusion Experiment

	Urinary excretion rate[a] (mg/hr, mean ± SD)	
Time (hr)	Hydroxymethyltolbutamide	Carboxytolbutamide
1	0.28 ± 0.18	0.31 ± 0.06
2	2.13 ± 0.63	3.07 ± 1.44
3	4.07 ± 1.63	7.16 ± 3.97
4	5.67 ± 1.02	12.26 ± 3.56
5	7.30 ± 0.86	15.99 ± 1.70
6	7.69 ± 1.24	18.58 ± 4.32
7	7.35 ± 0.45	20.73 ± 2.27
8[b]	7.15 ± 0.78	20.50 ± 2.65
9	4.45[c] ± 0.63	10.97 ± 3.74
10	5.19[d] ± 0.83	18.61 ± 4.27

[a]Values for five rabbits, expressed as mg/hr of tolbutamide.
[b]Sulfaphenazole was given intravenously immediately after collection of the 8-hr urine sample.
[c]Significantly slower than the rate at 8 hr ($p < 0.001$).
[d]Significantly slower than the rate at 8 hr ($p < 0.01$).

levels do not change significantly, although both are much lower than that of TB. The increase in CTB plasma levels after SP administration represents more support for the inhibitory effect of SP on the excretion of CTB. On the other hand, the fact that HTB blood levels remain unchanged before and after SP administration seems inexplicable from the urine data. This discrepancy, however, may be explained by using the pharmacokinetic model of TB proposed in previous work from this laboratory (8). SP could

Table V. Mean Plasma Levels of Metabolites of Tolbutamide in the Infusion Experiment

	Plasma level[a] (mg %, mean ± SD)	
Time (hr)	Hydroxymethyltolbutamide	Carboxytolbutamide
8[b]	1.18 ± 0.34	0.80 ± 0.11
8.5	1.02 ± 0.42	1.50[c] ± 0.31
10	0.77 ± 0.48	1.90[d] ± 0.56

[a]Values for four rabbits, expressed as mg % of tolbutamide.
[b]Sulfaphenazole was given intravenously immediately after collection of the 8-hr plasma sample.
[c]Significantly higher than the level at 8 hr ($p < 0.01$).
[d]Significantly higher than the level at 8 hr ($p < 0.02$).

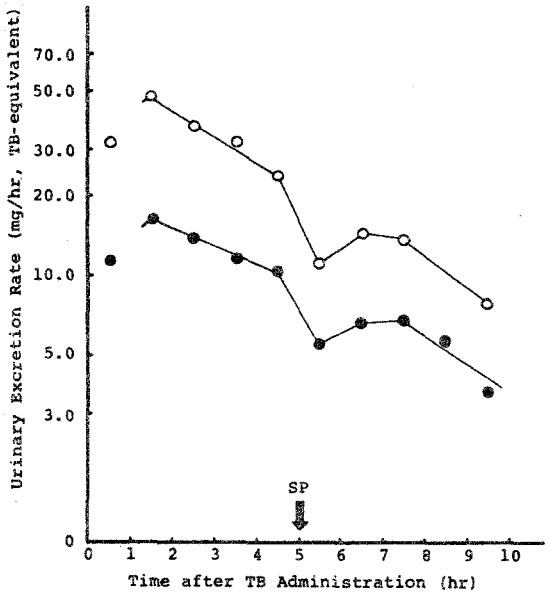

Fig. 4. Urinary excretion rates of HTB (●) and CTB (○) after an intravenous bolus of TB (150 mg/kg) and a subsequent intravenous bolus of SP (150 mg/kg).

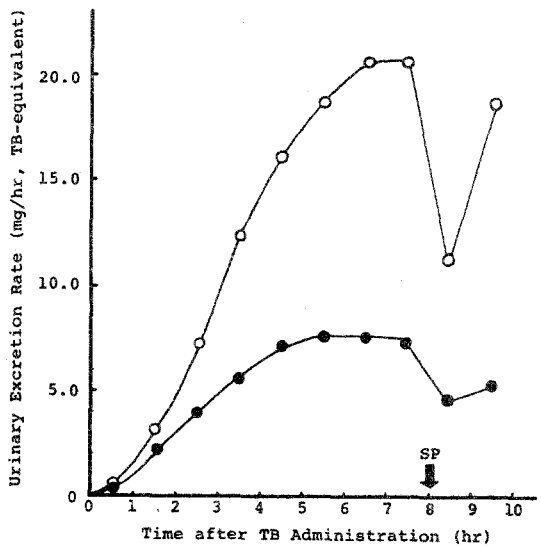

Fig. 5. Urinary excretion rates of HTB (●) and CTB (○) after a constant-rate infusion of TB (50 mg/hr) and a subsequent intravenous bolus of SP (150 mg/kg).

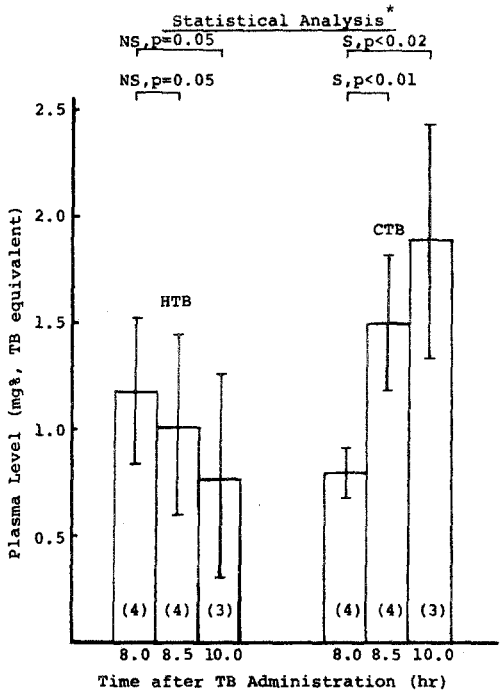

Fig. 6. Plasma levels of HTB and CTB after a constant-rate infusion of TB (50 mg/hr) and a subsequent intravenous bolus of SP (150 mg/kg). SP was given immediately after collection of the 8-hr plasma sample. The figures in parentheses indicate the numbers of determinations, and the vertical bars show the SD values for the mean levels. *Student's *t* test was used. NS, Not significant; S, significant.

be considered to have no effect on the transfer of HTB from blood to the hypothetical compartment, but it could inhibit the transfer of HTB from the compartment to urine. It may be possible to calculate a rough estimate of the renal clearance of CTB before and after SP administration using the excretion rates and plasma levels obtained from infusion experiment shown in Tables IV and V, respectively. The estimated renal clearance of CTB was 31 ml/min at 8 hr[4] (before SP administration) and 12 ml/min at 8.5 hr (after SP administration). These calculations are very rough, but there is no doubt that there is about a 2.5-fold reduction in the renal clearance of CTB in the presence of SP. It is not likely that the same thing takes place with the HTB

[4]The excretion rate of CTB at 8 hr has been estimated at about 15 mg/hr from the rate plot given in Fig. 5.

Table VI. Effect of Tolbutamide and Sulfaphenazole on Blood Sugar Levels Observed After Intravenous Injection[a]

A. Control level (10 min–2 hr before tolbutamide administration)	133.4 ± 40.4 (9)[b]
B. Level after tolbutamide administration (0.5–5 hr after the administration)	143.5 ± 55.4 (21)
C. Level after sulfaphenazole treatment[c] (0.5–5 hr after the treatment)	88.1 ± 33.5 (14)

Statistical analysis
B vs. A: nonsignificant ($p > 0.05$)
C vs. A: significant ($p < 0.01$)
C vs. B: significant ($p < 0.01$)

[a]Four rabbits were used and the blood samples were taken at various times during the intervals indicated; the blood sugar level is expressed as mg % of glucose (mean ± SD).
[b]The figures in parentheses indicate the numbers of determinations.
[c]Sulfaphenazole was given intravenously 5 hr after the administration of tolbutamide.

since its level drops while that of CTB increases. In spite of the limited accuracy of the analytical method presently utilized for the metabolites in plasma, it can be concluded that CTB is undergoing an active excretion process and that SP also is actively excreted and could be competing with the renal excretion of CTB.

Effect of SP on the Hypoglycemic Action of TB

The hypoglycemic effect of TB in rabbits was examined before and after SP administration, and the results are shown in Table VI. The blood sugar levels after treatment with both TB and SP are significantly lower than the levels of the control and TB-treated rabbits. Although the blood sugar level of TB-treated rabbits does not show a significant difference in relation to that of man and other animals, this is probably due to the experimental conditions of starving and restraining the animals. From the results of this study, it was confirmed that SP administration undoubtedly enhances the hypoglycemic action of TB in rabbits.

Hazards of Extrapolation from Animals to Man

In this study, two independent effects of SP on TB disposition in rabbits have been identified: (1) it may function as displacing agent for TB from the plasma protein, and (2) it is probably a competitive inhibitor of the active excretion process for CTB.

A considerable body of evidence in this study suggests that the effects of concurrent administration of SP on TB disposition in rabbits are markedly

different from those reported previously in man. When the mechanisms involved in a particular drug–drug interaction which clinically is known to cause a severe adverse reaction are studied, the use of laboratory animals is advisable. However, based on the data reported here for the rabbit, it must be emphasized that there are possible interspecies variations in the underlying mechanisms even for a given drug–drug combination. This important consideration must be taken into account in the extrapolation of animal data to the human in drug interaction studies.

REFERENCES

1. M. Kristensen and L. K. Christensen. Drug induced changes of the blood glucose lowering effect of oral hypoglycemic agents. In A. Loubatieres and A. E. Rengold (eds.), *Pharmacokinetics and Mode of Action of Oral Hypoglycemic Agents*, Proceedings of the Third Capri Conference, April 2–3, 1969, Casa Editrice "Il Ponte," Milan, 1969, pp. 116–136.
2. S. B. Matin, J. Karam, and M. Rowland. Kinetics of drug–drug interaction: Tolbutamide–sulfaphenazole interaction in man. *J. Pharmacokin. Biopharm.* (submitted).
3. L. K. Christensen, L. M. Hansen, and M. Kristensen. Sulfaphenazole induced hypoglycemic attacks in tolbutamide treated diabetics. *Lancet* **2**:1298–1301 (1963).
4. E. Schulz and F. H. Schmidt. Abbauhemmung von Tolbutamid durch Sulfaphenazol beim Menschen. *Pharmacol. Clin.* **2**:150–154 (1970).
5. M. Rowland and S. B. Matin. Kinetics of drug–drug interactions. *J. Pharmacokin. Biopharm.* **1**:553–567 (1973).
6. J. Judis. Binding of sulfonylureas to serum protein. *J. Pharm. Sci.* **61**:89–93 (1972).
7. L. Skovsted, J. M. Hansen, M. Kristensen, and L. K. Christensen. Inhibition of drug metabolism in man. In P. L. Morselli, S. Garattini, and S. N. Cohen (eds.), *Drug Interactions*, Monographs of the Mario Negri Institute for Pharmacological Research, Milan, Raven Press, New York, 1974, pp. 81–90.
8. J. Shibasaki, R. Konishi, T. Morishita, and T. Ueki. Drug absorption, metabolism, and excretion. IX. Some new aspects of pharmacokinetics of tolbutamide in rabbits. *Chem. Pharm. Bull.* **21**:1754–1763 (1973).
9. J. Shibasaki, R. Konishi, T. Ueki, and T. Morishita. Drug absorption, metabolism, and excretion. VIII. Determination of tolbutamide and its metabolites in blood and urine. *Chem. Pharm. Bull.* **21**:1747–1753 (1973).
10. M. Somogyi. Notes on sugar determination. *J. Biol. Chem.* **195**:19 (1952).
11. H. Spingler. Ueber eine Moeglichkeit zur Colorimetrischen Bestimmung von *N*-(4-Methyl-Benzolsulfonyl)-*N'*-Butyl-Harnstoff in Serum. *Klinische Wochenschr.* **35**: 533–535 (1957).