[Chem. Pharm. Bull. 26(2) 387—393 (1978)]

UDC 615.217.34.033.076.9 : 546.26.02.14.08

Intestinal Absorption of a New Anticholinergic Agent, Timepidium Bromide. II.[1] Enhancement of Absorption

HAJIME TAMAKI[2a] and TERUHIKO MESHI[2b]

Biological and Chemical Research Laboratories, Tanabe Seiyaku Co., Ltd.[2]

(Received May 4, 1977)

Enhancement of absorption of a quaternary anticholinergic agent, timepidium bromide (TB), was studied *in vivo* and *in situ* in rats. Intraduodenally administered citric acid markedly increased the amount of TB absorbed, but orally administered citric acid did only slightly. The difference in absorption of TB between oral and duodenal administration of citric acid was assumed to be due to the fact that citric acid delayed gastric emptying. It was found that the absorption of TB from *in situ* intestinal loop varied with drug concentration, the greater the concentration the greater the absorption. In the presence of high concentrated solution of tertiary amine of TB, diphemanil methylsulfate or prifinium bromide, which are structural analogues of TB, the absorption of TB was markedly enhanced. This increased absorption of TB was unrelated to the activity of the additives as an anticholinergic agent.

Keywords——intestinal absorption; quaternary ammonium compounds; timepidium bromide; hyoscine-N-butylbromide; diphemanil methylsulfate; citric acid; gastric emptying; organic acid; enhancement of absorption; anticholinergic agent

In a previous study on various factors influencing intestinal absorption of timepidium bromide (TB) which is a quaternary anticholinergic agent, mucin, intestinal mucous material and bile were found to decrease the absorption of TB from *in situ* intestinal loops whereas gastric juice and various acids increased that.[1] Among the organic acids studied, citric acid increased most greatly the absorption of TB. Consequently, studies were designed to determine the facilitating effect of citric acid on gastrointestinal absorption of TB. However, when citric acid was administered orally together with TB the facilitating effect of citric acid on gastrointestinal absorption of TB was, unexpectedly, not so great as compared with that when administered intraduodenally.

Another approach to the modification of the rate of the gastrointestinal absorption of TB was made as follows. It is generally assumed that poor absorption of quaternary ammonium compounds is due to their ionized character, probably because of anionic groups present in intestinal mucous membranes retain the cations of quaternary ammonium compounds by electrostatic bonding. It might, therefore, be possible to administer, together with the anticholinergic agent, a quaternary which was physiologically inert but which could effectively compete at sites of loss in the gut, thereby resulting in greater availability for absorption of free unabsorbed active quaternary. From the same point of view, the effect of high concentration of the active quaternary might be expected to increase degree of absorption. This paper reports on the results of those studies.

Experimental

^{14}C-TB and Reagents——The preparation of ^{14}C-TB was described in the previous paper.[1] All of the chemicals employed in this study were reagent grade unless otherwise specified.

1) Part I: T. Meshi and H. Tamaki, *Chem. Pharm. Bull.* (Tokyo), **26**, 379 (1978).
2) Location: *Toda-shi, Saitama, 335, Japan*; *a*) To whom reprint requests should be sent; *b*) Present address: *Department of Pharmacology, Faculty of Pharmaceutical Science, Okayama University, Okayama, 700, Japan.*

Mode of Administration——Male Sprague–Dawley rats weighing between 180—200 g were selected for use in this study. Food was withheld from the animals for 16 hr prior to experimentation but water was allowed *ad libitum*. The animals were housed in cages having wide mesh floors to prevent coprophagy. A dose of 3 mg of ^{14}C-TB dissolved in 2 ml of water or 0.15 M citric acid was orally administered by means of a stomach tube. For intraduodenal administration, the animals were anesthetized approximately 1 hr prior to surgery with *i.p.* urethane (1 g/kg). Laparotomy was performed through a midline incision and the common bile duct was ligated. A dose of 1 mg of ^{14}C-TB dissolved in 1 ml of water or 0.15 M citric acid was injected into the duodenum. After 1 or 2 hr, about 5 ml blood was collected from the heart and then whole gastrointestinal tract was removed.

Determination of ^{14}C-TB in Blood and Gastrointestinal Tract——To 4 ml of blood was 16 ml of 75% alcohol added and then centrifuged. Ten ml of the supernatant was transferred to a 50 ml centrifuge tube and alkalinized with 2 drops of 20% NaOH. ^{14}C-TB and its metabolites were extracted with an equal volume of $CHCl_3$. An aliquot of the $CHCl_3$ extract was determined for ^{14}C, and then analyzed for unchanged ^{14}C-TB by thin-layer chromatography (TLC) in a solvent system of $CHCl_3$–MeOH–AcOH (75 : 20 : 5).[3] The gastrointestinal tract was slit open for gastrointestinal contents to be washed out in 40 ml of cold physiological saline. The gastrointestinal walls were homogenized with 40 ml of MeOH. An aliquot of the intestinal contents and the supernatant of homogenates were analyzed for unchanged ^{14}C-TB in the same manner as described for blood samples. The degree of the absorption of ^{14}C-TB was calculated from the difference between the amount of the drug administered and that recovered from the intestinal tissue and contents.

Measurement of Gastric Emptying Rate——The rate of gastric emptying was determined by the rate of transition of phenol red.[4] The rats were given 2 mg/2 ml of phenol red dissolved in distilled water or 0.15 M acid solution. The rats were killed 30 min after administration, and the stomach, small and large intestines were taken off and homogenized with 20 volumes of 0.1 N NaOH. The homogenates were centrifuged, and aliquots of the supernatant were read against 0.1 N NaOH at 540 mμ in a Hitachi-Perkin spectrophotometer.

Absorption from *in Situ* Intestinal Loops——Details of the absorption technique and procedure employed in ^{14}C-TB analysis were presented in the previous paper.[1] The multiple-loop preparation was carried out according to the method of Levine and Pelikan.[5]

Results

Effect of Citric Acid on *in Vivo* Absorption of TB

The gastrointestinal absorption of TB during 1 and 2 hr after oral administration of TB with or without citric acid, is shown in Table I. The amounts of TB absorbed during 1 and 2 hr was slightly greater in the presence of citric acid than in its absence. The small enhancement of absorption was also shown by the blood levels of TB, which was slightly increased in animals that had received citric acid. Most of the stomach containing citric acid were found to be distended with fluid at the time of excision while the control stomach was not.

Table I. Effect of Citric Acid on Gastrointestinal Absorption of ^{14}C–TB after Oral Administration

Time after administration	Control		0.15 M citric acid	
	% of dose absorbed	Blood level of ^{14}C–TB (ng/ml)	% of dose absorbed	Blood level of ^{14}C–TB (ng/ml)
1 hr	4.6±0.8	3.3±0.5	6.2±2.5	3.9±0.6
2 hr	5.7±2.7	5.8±2.1	11.2±3.5	10.5±3.4

A dose of 3 mg of ^{14}C–TB was dissolved in 2 ml of water or 0.15 M citric acid and administered orally to rats. One or two hour after administration, blood specimens (about 5 ml) were withdrawn from the heart and then whole gastrointestinal tracts were removed for quantitation of residual ^{14}C–TB in the gut. Results are mean±S.D. from 4 rats.

3) T. Meshi, S. Nakamura, and T. Kanno, *Chem. Pharm. Bull.* (Tokyo), **21**, 1709 (1973).
4) R. Kato, A. Tanaka, K. Onoda, and Y. Omori, *Jpn. J. Pharmacol.*, **19**, 331 (1969).
5) R.R. Levine and E.W. Pelikan, *J. Pharmacol. Exp. Ther.*, **131**, 319 (1961).

The intestinal absorption of TB during 1 hr after intraduodenal administration of TB with and without citric acid, is shown in Table II. The absorption of TB was markedly increased in the presence of citric acid. When administered alone, TB was absorbed to the extent of 6.5 and 11.0% in bile duct-nonligated and ligated rats respectively, and when administered together with citric acid, the degree of absorption rose to 26.6 and 43.4% respectively. Moreover, citric acid-treated animals had blood levels of TB 6 and 10 times higher than those of control animals. In the presence of citric acid, the degree of absorption and blood level of TB were significantly greater in bile duct-ligated than in bile duct-nonligated rats, but in its absence those were not significantly greater in bile duct-ligated rats.

Table II. Effect of Citric Acid on Intestinal Absorption of ^{14}C–TB in Bile Duct Ligated and Nonligated Rats after Intraduodenal Administration

Bile duct treatment	Control		0.15 M citric acid	
	% of dose absorbed	Blood level of ^{14}C–TB (ng/ml)	% of dose absorbed	Blood level of ^{14}C–TB (ng/ml)
Ligated	11.0±3.0	3.5±2.2	43.4±4.8[a)]	37.7±8.4[a)]
Nonligated	6.5±2.5	3.2±2.1	26.6±1.1[a)]	21.1±6.6[a)]

A dose of 1 mg of ^{14}C–TB was dissolved in 1 ml of water or 0.15 M citric acid and injected into the duodenum of rats. One hour after injection, blood specimens (about 5 ml) were withdrawn from the heart and then whole gastrointestinal tracts were removed for quantitation of residual ^{14}C–TB in the gut. Results are mean±S.D. from 4 rats.
a) Significantly different ($p<0.01$) from the comparable value for control.

Effect of Acids on Gastric Emptying Rate

The distribution of phenol red in gastrointestinal tract of rats at 30 min after oral administration of phenol red with or without acids, is shown in Table III. When phenol red was administered alone, only about 10% of the administered dose was recovered from the stomach. However, when administered together with citric acid, the amount of phenol red remaining in the stomach rose to about 40%. Similarly the administration of other acids inhibited the gastric emptying rate. The effect of acids on gastric emptying rate was the greatest in citric acid, middle in malic and glucuronic acid, the lowest in acetic acid. These results were consistent with the recent findings by Blum, *et al.*[6] that the inhibition of gastric emptying rate increased with rising number of carboxylic groups of the acid and its molecular weight.

Table III. Effect of Various Organic Acids on Gastric Emptying Rate in Rats

Additive	Stomach	Small and large intestine	% phenol red absorbed
Control	10.7±3.3	83.0± 4.4	6.3±3.4
TB (5 μM)	16.4±2.5	78.8± 6.4	4.8±4.3
Citric acid (0.3 mM)	42.2±9.5[a)]	43.8±11.4[a)]	14.0±5.7
Malic acid (0.3 mM)	29.2±8.6[a)]	55.9±13.1[a)]	14.9±8.6
Glucuronic acid (0.3 mM)	30.6±3.4[a)]	54.0± 4.6[a)]	15.4±4.2[a)]
Acetic acid (0.3 mM)	19.0±3.4[a)]	67.8± 7.1[a)]	13.2±5.4

Thirty min after an oral administration of 2 mg/2 ml of phenol red dissolved in distilled water or 0.15 M acid solution, rats were sacrificed, and the stomach, small and large intestines were taken off. Results were expressed as the mean percentage of the dose in 4 rats with S.D.
a) Significantly different ($p<0.05$) from comparable value for control.

6) A.L. Blum, J. Hegglin, G.J. Krejs, F. Largiader, H. Säuberli, and P. Schmid, *J. Physiol.* (London), **261** 285 (1976).

It is known that phenol red is poorly absorbed from the gastrointestinal tract.[7] In the present study, phenol red is absorbed to the extent of 6.3% in 30 min. Acids slightly increased the absorption of phenol red.

Absorption of TB from *in Situ* Gastrointestinal Loops

The circulatory uptake, which refers to material taken into blood or lymphatic fluid, showed that the amount of the drug absorbed from each loop during 2 hr was relatively constant level. As shown in Table IV, the middle loop absorbed slightly more than the loop closest to the pylorus, which absorbed more than the distal. The stomach absorbed to the same extent as the distal loop did. The addition of citric acid significantly increased the amount of TB absorbed from each loop as compared with each control.

Table IV. Effect of Citric Acid on Absorption of TB from Various Posititions of Gut

Position	Control		0.1M citric acid	
	Circulatory uptake % of dose/loop	Tissue binding % of dose/loop	Circulatory uptake % of dose/loop	Tissue binding % of dose/loop
Stomach	9.4±1.6	7.4±4.0	25.9±4.2[a]	5.6±2.2
Proximal of small intestine	11.0±5.8	12.8±5.8	38.7±5.2[a]	10.4±2.4
Middle of small intestine	14.5±3.9	16.7±3.8	40.1±6.9[a]	5.6±1.2[a]
Distal of small intestine	9.4±1.1	9.4±3.8	24.9±3.0[a]	4.6±0.6[a]

All studies are for 2-hour periods. A dose of 0.2 mg of ^{14}C–TB with or without 0.1 M citric acid was injected in a total volume of 0.5 ml. Results were expressed as the mean percentage of the dose in 3 to 4 rats with S.D.
a) Significantly different ($p<0.05$) from comparable value for control.

Effect of Inert Quaternary on Absorption of TB from *in Situ* Intestinal Loops

When TB was administered alone in the intestinal loops (middle of small intestine), this drug was absorbed to the extent of only 2% during 30 min. On the other hand, when administered together with carnitine hydrochloride or betaine hydrochloride, the degree of absorption rose to 8 and 26% respectively, as shown in Table V. However, free carnitine and betaine had no facilitating effect on the circulatory uptake of TB. Choline chloride and thiamine hydrochloride had no effect on the control level of the circulatory uptake.

Table V. Effect of Various Quaternary Ammonium Compounds on Absorption of ^{14}C–TB from *in Situ* Intestinal Loops

Additive	Molarity of additive	pH of solution injected	Circulatory uptake % of dose/loop	Tissue binding % of dose/loop
Control		5.8	1.9±1.2	9.3±3.0
Carnitine HCl	0.1M	2.5	7.9±3.0[a]	7.4±1.8
Carnitine (free)	0.1M	7.2	2.5±0.8	6.2±1.3
Betaine HCl	0.1M	1.6	26.5±2.4[a]	4.7±0.9[a]
Betaine (free)	0.1M	5.9	3.4±1.3	6.8±1.9
Choline chloride	0.1M	6.7	2.9±1.7	7.2±1.2
Thiamine HCl	0.1M	3.0	3.5±1.6	10.4±1.7

All studies are for 30 min periods. A dose of 0.2 mg of ^{14}C–TB with or without additive was injected in a total volume of 0.5 ml. Results were expressed as the mean percentage of the dose in 4 rats with S.D.
a) Significantly different ($p<0.05$) from the comparable value for control.

7) *a*) R.R. Levine, *J. Pharmacol. Exp. Ther.*, **131**, 328 (1961); *b*) K. Kakemi, H. Sezaki, R. Konishi, T. Kimura, and M. Murakami, *Chem. Pharm. Bull.* (Tokyo), **18**, 275 (1970).

Effect of Concentration on Intestinal Absorption of TB

The mean circulatory uptake and standard deviation in mg absorbed for each dose level are graphically represented in Fig. 1. When the volume of the drug solution injected into loop was 0.5 ml, the circulatory uptake during 30 min was almost constant at 2—4% of the dose per loop over the entire dosage range studied although the standard deviation was great. However, when the volume of the drug solution was reduced to 0.1 ml, the absolute amount absorbed was not directly related to the doses, and increasing amounts of TB were absorbed with increasing doses. The points corresponding to doses of 0.3 and 0.4 mg, respectively, seemed likely to be inflection points since the % circulatory uptake (4.5% at 0.3 mg and 14.9% at 0.4 mg) was markedly different between doses of 0.3 and 0.4 mg. It seemed that the dose-absorption curve might be divided into two major straight line segments, even though the total curve might be describable as a curvilinear function of dose: the first segment defined by the points between doses of 0.1 and 0.3 mg, and the second between 0.3 and 1.6 mg.

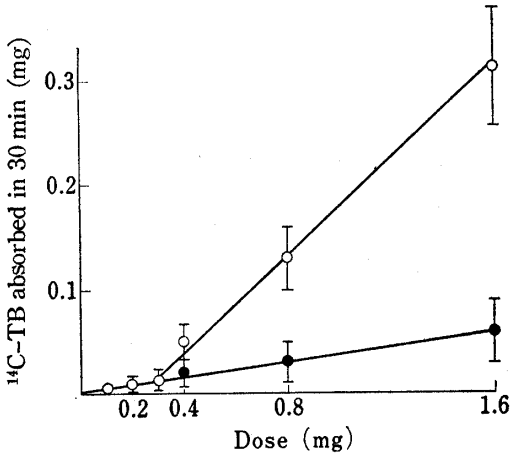

Fig. 1. Dose-Absorption Curve for ^{14}C-TB
All studies are for 30 min periods. Each dose was injected in a volume of 0.1 and 0.5 ml. Each point represents the mean absorption (mg) in 4 animals with S.D.
○: 0.1 ml, ●: 0.5 ml.

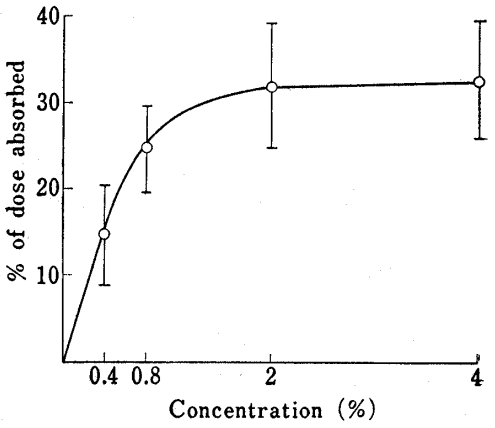

Fig. 2. Effect of Concentration on Absorption of ^{14}C-TB from *in Situ* Intestinal Loops
All studies are for 30 min periods. A dose of 4 mg of ^{14}C-TB was injected in various volumes of 0.1—1.0 ml. Each point represents the mean absorption (% of dose) in 4 animals with S.D.

Effect of concentration on absorption of TB is shown in Fig. 2. The circulatory uptake curve might be describable as hyperbolic function of the concentration. When the concentration was raised to more than 2%, the % circulatory uptake remained constant at about 30% of the dose.

Effect of Anticholinergic Drugs and Their Related Compounds on Absorption of TB

In the presence of some of anticholinergic drugs, absorption of TB was markedly increased, as shown in Table VI. When high concentration (0.1 M) of prifinium bromide (PB) or diphemanil methylsulfate (DMS), was administered together with ^{14}C-TB, the % dose of ^{14}C-TB absorbed in 30 min was increased about 20 fold as compared with that when ^{14}C-TB was administered alone, whereas hyoscine-N-butylbromide (HB) had no such a facilitating effect. Atropine sulfate (Atr) enhanced moderately absorption of TB. Tertiary amine of TB also enhanced absorption of TB to the same extent as PB and DMS did. Methacholine, a cholinergic drug, had no effect on the control level of the circulatory uptake of TB and on high level of the circulatory uptake observed when high concentration of TB was used.

Table VI. Effect of Various Drugs on Absorption of ^{14}C–TB from *in Situ* Intestinal Loops

Additive	Molarity of additive	Circulatory uptake % of dose/loop	Tissue binding % of dose/loop
Control (^{14}C–TB)	(0.005M)	1.9±1.2	9.3± 3.0
Methacholine	0.1M	3.1±1.5	8.4± 1.7
HB	0.1M	3.9±3.0	20.5±10.5
PB	0.1M	40.4±7.5[a)]	12.5± 2.3
PB	0.01M	11.9±4.1[a)]	20.5± 5.8[a)]
DMS	0.1M	36.1±8.4[a)]	13.8± 2.7
DMS	0.01M	9.2±3.3[a)]	16.7± 4.1[a)]
TB	0.1M	31.6±8.0[a)]	6.4± 0.7
TB and Methacholine	0.1M and 0.1M	29.8±6.4[a)]	6.1± 1.3
Atr	0.1M	21.4±1.4[a)]	21.6± 7.0[a)]
Tertiary amine of TB	0.1M	33.8±8.3[a)]	11.5± 1.0

All studies are for 30 min periods. A dose of 0.2 mg of ^{14}C–TB was injected with or without additive in a total volume of 0.1 ml. Results were expressed as the mean percentage of the dose in 4 rats with S.D.
a) Significantly different ($p<0.05$) from the comparable value for control.

Discussion

When citric acid was administered duodenally together with ^{14}C–TB, the degree of absorption and blood levels of ^{14}C–TB was markedly increased as compared with controls. When administered orally, however, the increase in absorption and blood levels was small. Moreover, when citric acid was administered orally, the stomach was found to be distended with fluid at the time of excision. These facts led us to study the influence of citric acid on gastric emptying rate. The results of the present study showed that citric acid markedly delayed the rate of gastric emptying. This observation was in good agreement with the findings that various organic acids including citric acid slowed the gastric emptying in human subjects and experimental animals.[6,8)] It seems that there are two reasons why the retarded gastric emptying inhibits the facilitating effect of citric acid on absorption of TB; one is that absorption of TB occurs predominantly in the small intestine and the other is that facilitating effect of citric acid is canceled by the bile. The stomach was shown to absorb TB to the same extent as the distal loop of the small intestine did. However, it seems reasonable to consider that TB is absorbed more largely from the small intestine than the stomach, since the former has a much larger relative surface area for absorption. On the other hand, it was shown in the previous study that citric acid had facilitating effect only when added in relatively high concentrations (0.05 M) and at relatively low pH (2.2). If the rate of gastric emptying is delayed, the concentration of citric acid is diluted more largely with bile and digestive juice, and the pH of it is raised higher by neutralizing with bile and pancreatic juice.

Recently, Nimmo, *et al.*[9)] showed that propantheline bromide delayed gastric emptying and markedly slowed the absorption of paracetamol in six convalescent hospital patients while the absorption of it was accelerated by metoclopramide, a drug which stimulates gastric emptying. Like propantheline bromide, TB has inhibitory effects on the motor activity of the stomach and small intestine.[10)] However, the present study showed that effect of TB on gastric emptying was small.

It has been shown in the previous study[1)] that bile, mucin and mucous material significantly decrease the amount of TB absorbed from intestinal loops and that they, *in vitro*, form

8) J.N. Hunt and M.T. Knox, *J. Physiol.* (London), **201**, 161 (1969).
9) J. Nimmo, R.C. Heading, P. Tothill, and L.F. Prescott, *Br. Med. J.*, **1**, 587 (1973).
10) H. Tamaki, M. Tanaka, S. Murata, S. Harigaya, and A. Kiyomoto, *Jpn. J. Pharmacol.*, **22**, 685 (1972); H. Tamaki, T. Ikeo, S. Harigaya, and H. Nakajima, *Jpn. J. Pharmacol.*, **23**, 391 (1973).

quaternary salt complexes with TB. It might, therefore, be possible to administer, together with TB, a quaternary which was physiologically inert but which could effectively compete at sites of loss in the gut, thereby resulting in greater availability for the absorption of free unadsorbed TB. However, unexpectedly, choline chloride, thiamine hydrochloride, free carnitine and betaine had no effect on the absorption of TB from intestinal loops although carnitine hydrochloride and betaine hydrochloride significantly increased the absorption of TB. The fact that carnitine hydrochloride and betaine hydrochloride increased the absorption of TB, seems to be due to their acidic mediums, since it has been demonstrated that the acidic medium has facilitating effect on the absorption of the quaternaries.[1,7a,11]

It should be noted that the degree of the absorption varied with the drug concentration, the greater the concentration the greater the degree of the absorption. When the volume of the drug solution injected into the intestinal loop was 0.5 ml, the dose-absorption curve was almost linear. This relation suggested that the absorption of TB occurs by simple diffusion. However, when the volume of the drug solution was reduced to 0.1 ml, it seemed that the dose-absorption curve might be divided into two segments. The latter case was in agreement with the results of Levine and Pelikan[5] that the dose-absorption curve of benzomethamine, a quaternary ammonium drug, was a composite curve and not a single, linear function through the entire range of doses used. These facts suggested that more than one mechanism of transfer of the quaternaries across the gut wall may be involved in the absorption of the quaternaries. Indeed, it was found that high concentration of DMS and PB, structural analogues of TB, enhanced the absorption of ^{14}C–TB to the same extent as observed when high concentration of TB was used. From these facts, it seems likely that these increased absorption may be ascribed to an increased permeability induced by TB and its structural analogues. Common chemical structures that TB, DMS and PB have in their molecules, are two aromatic rings, a heterocyclic ring containing cationic nitrogen, and a double bond bridge linking aromatic rings and heterocyclic ring. Presumably, this basic structure may change the permeability of the mucous membrane. The fact that tertiary amine of TB significantly enhanced the absorption of ^{14}C–TB indicates that the presence of a quaternary ammonium group in its molecule is not necessary for the enhancement of the absorption of ^{14}C–TB.

In the present study, no definite information was available pertaining to the mechanism of the enhancement of the absorption of TB. However, it is clear that anticholinergic activity of TB is unrelated to facilitating effect of TB on absorption, since the addition of methacholine did not inhibit facilitating effect of TB on the absorption of itself.

Acknowledgement The authors wish to express their deep gratitude to Dr. K. Abe, Director of Biological and Chemical Research Laboratories, Dr. A. Kiyomoto, Dr. H. Nakajima and Dr. S. Harigaya for their interest and encouragement. The technical assistance of Mrs. K. Joh is gratefully recognized.

11) R.H. Reuning, B.L. Ross, B.J. Schoemaker, and S.S. Watson, *Pharmacologist*, **13**, 195 (1971).