herausgenommen, mit 2 ml N HCl und dann 1 ml 1 Mol KJ versetzt und 10 Min. im Dunkeln stehengelassen. Das ausgeschiedene Jod wurde mit 0.01N $Na_2S_2O_3$ titriert. Das Resultat ist in folgender Tabelle gezeigt.

Dem Nationalfonds zur Förderung der wissenschaftlichen Forschung (Unterrichtsministerium) danken wir bestens für die Unterstützung dieser Arbeit. Wir danken Frl. H. Yamanouchi und K. Hayashi sowie N. Kurosawa an diesem Laboratorium für die Ausführung der Mikroanalysen sowie die Aufnahme der IR-Spektren.

Zusammenfassung

Es wird über die Synthese sowie die Konfigurationsbestimmung der vier Isomeren des zum Progesteron gehörigen 14,15-Diols geschrieben.

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[Chem. Pharm. Bull. 11 (10) 1282 ~ 1290]

UDC 615.7[616.3-002.44-085]-092

206. Keijiro Takagi, Yutaka Kasuya, and Kazuo Watanabe : *1 Studies on the Drugs for Peptic Ulcer. Pharmacological Studies on the Effects of Synthetic Antispasmodics mixed with Antacid.

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Now a large number of drugs are used for treatment of peptic ulcer, and among them synthetic anticholinergic antispasmodics have been prescribed preferably since methantheline appeared in 1950. Antispasmodics are often administered to patients in combination with antacids and their mixture is frequently prepared for oral administration. This paper deals with two problems. One is to know whether the synthetic antispasmodics are stable or they diminish their therapeutic effects when they are mixed with the antacid, which is a strong base and powerful adsorbent. For this purpose we employed biological assay methods. There are, of course, some methods to estimate the degree of decomposition of drugs by chemical methods, but the former is superior to the latter in this research because it needs only minute samples and it indicates directly the effects of drugs on living organs. Actually it is said to be difficult to assay the contents of antispasmodics by the chemical way when 1~2 mg. of them are mixed and adsorbed in 1 g. of synthetic aluminum silicate. Another purpose is to search the suitable biological assay method for estimating the effects of remedies for peptic ulcer. Although there are many experimental data on their effects on gastric secretion or on isolated gastrointestinal tract, we can scarcely find the experimental report in which the potency ratio of drugs preventing or healing gastric ulcer can be compared directly.

Of some methods to produce peptic ulcer on experimental animals, Shay's procedure[1] is commonly employed for these purposes. In this method, the stomach of a rat is ligated on pylorus after some periods of starvation. With this simple procedure acute peptic ulcers appear consistently only after 16~18 hours. In view of etiology and other

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1) H. Shay, S. A. Komarov, S. Fels, D. Merenze, M. Gruenstein : Gastroenterology, **5**, 43 (1945).

characteristics, Shay rat ulcer is said to resemble human acute peptic ulcer. We examined the experimental conditions of Shay's method and modified them to fit our purpose to compare the effects of various drugs used for peptic ulcer.

Under this modified condition, the effects of a few drugs and of combined administration of antispasmodics with antacid were tested.

Experimental

1) **Preparation of Specimens**——Three kinds of mixture were prepared, which contained 1 mg. of atropine methobromide (Atr. MeBr), benactyzine methobromide (Ben. MeBr) or 1 mg. of atropine sulfate (Atr.) in 1 g. of synthetic aluminum silicate, and examined the anticholinergic activities of these mixtures after they had been kept in moist container for over three months in room temperature. The synthetic aluminum silicate was selected as antacid, because it is convenient for separation from various extracting solvents, and actually in clinical use.

The solvents used for extraction were distilled water Tyrode's solution, and 0.1*N* HCl solution.

2) **Assay with Isolated Intestines of Mice**——According to usual method using isolated small intestines of mice and acetylcholine as the stimulant (10^{-7} g./ml. in bath), the recoveries of antispasmodics from each sample were assayed comparing the spasmolytic activity of the test solution with that of the standard solution which contained known amounts of the drug. The test solutions were prepared as follows : a test mixture was shaken well with Tyrode's solution for 30 min. A part of the suspension was centrifuged and the precipitate of aluminum silicate was discarded.

These two solutions, the suspended mixture and its supernatant, were served as the test solutions. To know the degree of elution of the drug from the antacid into gastric juice, the supernatant of the suspension of the mixture, which was shaken well in 0.1*N* HCl at 26°, was also prepared.

3) **Assay with Mydriasis of Mice Eyes**——Mice weighing about 20 g. were selected and the diameters of their pupils under the normal condition were measured with magnify glass which has a built-in micrometer of 0.1 mm. The mice whose diameter of pupils were over 0.4 mm were omitted. The test animals were starved for about 12 hr. before the drugs were administered. At the begining of an experiment, the initial diameter was measured 3 times repeatedly, then the drug was administered by a stomach tube or by injection.

The diameter of pupils was measured every 5 min. for 1 hr., and the maximum response caused by the drug was determined. At the end of the experiment, the excess amount of atropine was injected intravenously and the maximum diameter was measured. We used the following equation to calculate the metameter of the mydriasis.

$$X=\{(m-I)/(M-I)\}\times 100$$

Selection of this metameter is based on the results shown in Fig. 1.

4) **Experimental Ulcer**——Male rats weighing about 160 g. were used. Before the operation, rats were starved for 78 hr. During these periods, all animals were divided in separated cages with raised bottom of wide wire mesh, and water was permitted ad libitum. Room temperature was kept at 22～23°. Operative procedures were carried out according to Shay's original report.[1] Under light ether anesthesia, the stomach was exposed and ligated at pylorus, and then abdominal wall was closed by interrupted sutures. After 19 hr. the abdominal incision was made again and the ligature was placed around the esophagus close to the diaphragm to isolate the stomach.

The isolated stomach was then opened along the greater curvature and was stretched on a board. After wiping its mucosa with saline, it was examined with magnifying glass. Gastric contents were taken into a graduated centrifuge tube. Almost all ulcers were found in forestomach and had round shape. Some times round or linear ulcers were found in glandular portion of the stomach. Blood coagulated on the surface of ulcer made it easy to find even a small one. The damage of mucosa was taken as a criterion of ulcer to distinguish it from a spot of haemorrhage. Ulcers were classified into five degrees by their diameters. Details of classification are shown in Table III. The gastric contents were centrifuged after the measurement of their volume and the supernatants were removed to titrate the free and the total acid concentration with 0.02*N* NaOH using Topfer's reagent and phenolphthaleine as indicator.

Results

(1) Stability of Antispasmodics mixed with Antacid

Spasmolytic activities of three drugs, atropine methobromide, benactyzine methobromide and atropine sulfate mixed with aluminum silicate were assayed by Magnus'

method using isolated small intestine of mouse. In a week after preparation of the mixtures, the recovery of each drugs was about 100%, but after that the activities of the mixtures came to diminish rapidly and in 10 days they fell to lower than 30% of the initial state.

Then the following experiments were performed to make clear whether this phenomenon was due to decomposition of drugs or due to adsorption of them by antacid, which was so firm that they could not be eluted out. Comparing the spasmolytic activity of suspension of the mixture in Tyrode's solution with that of the supernatant of this suspension separated by centrifuge, the former specimen displayed distinctly higher activity than the latter and the difference between them became smaller in proportion to the dilution of suspension. The relation between dilution and recovery of atropine from tne supernatant is demonstrated in Table I.

Table I. Recoveries of Atropine into Tyrode's Solution from the Mixture of Atropine Sulfate and Aluminum Silicate

Tyrode (ml.)	100	500	1,000	5,000	10,000	250,000
Recovery (%)	59	71	77	82	83	100

1 g. of mixture was extracted with 100~250,000 ml. of Tyrode's solution. Recovery was assayed by spasmolytic activity.

At the dilution of 250,000:1, spasmolytic activity of the supernatant was equal to that of the suspension and recovery ratios of both samples were 100% even six months after the preparation of the mixture. Considering these results, synthetic aluminum silicate does not seem to decompose atropine, but adsorbs it tightly.

(2) Elution of Drugs from the Antacid into Gastric Juice

To know the degree of the elution of adsorbed drug into gastric juice, recovery of drugs from the sample was estimated as follows. After shaking the mixture in 0.1*N* hydrochloric acid at 26° for 5 minutes, the suspension was centrifuged and its supernatant was separated. As shown in Table II recovery in acidic solution was higher than that in Tyrode's solution. At the dilution of 500:1, the recovery came to nearly 100%, and differences among three drugs could not be shown.

Table II. Recoveries of Some Antispasmodics from 0.1*N* HCl Extracts of their Mixture with Antacid

0.1*N* HCl (ml.)	100	500
Atropine	87.3 (%)	99.0 (%)
Atropine MeBr	83.8 〃	97.4 〃
Benactyzine MeBr	76.5 〃	93.8 〃

(3) Absorption of Atropine from the Gastrointestinal Tract

From the preceding experiments, it was demonstrated that antispasmodics were not decomposed by combination with the antacid, but it seemed probable that the absorption of drugs through gastrointestinal tract would be inhibited when they were administered by oral route in combination with antacid. To confirm this fact the response of mouse pupil to atropine was utilized.

Pulewka's method[2] is very convenient for the estimation of mydriatic activity of a drug, but there are some questions with regard to the metameter which indicates the mydriatic activity. The authors examined various metameters by statistical calculation, and decided to use the metameter II which had the smallest λ as shown in Fig. 1. Mydriasis caused by the old mixture was compared with that of the pure atropine

2) . Pulewka : Arch. Exptl. Pathol. Pharmakol., **168**, 307 (1932).

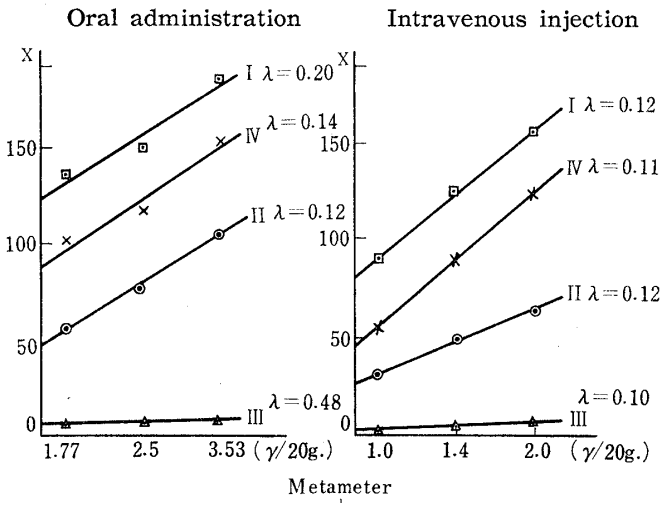

Fig. 1. Dose-response Line of Mydriatic Action of Atropine Sulfate and Comparision of various Metameters

I : $X=m$(1/100 mm.)

II : $X=\frac{m-I}{M-I}\times 100$

III : $X=\frac{m}{I}$

IV : $X=m-I$(1/100 mm.)

I : Initial diameter

m : Maximum diameter by test dose

M : Maximum diameter by excess atropine

solution and with that of the mixture which was prepared just before the experiment in the same ratio of atropine to synthetic aluminum silicate as the old mixture. From this experiment, absorption of atropine from gastrointestinal tract was known to be lowered by combination with the antacid as illustrated in Fig. 2. Difference between the old mixture and the fresh one, however, was very small, then we confirmed the view that the decomposition of atropine in the presence of the antacid would be rather slight.

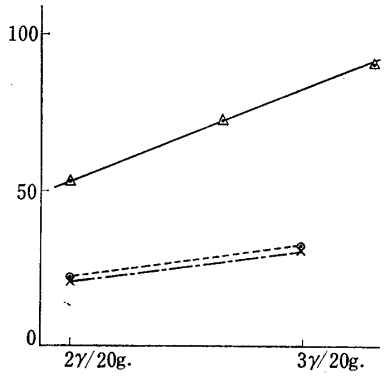

Fig. 2. Mydriatic Action of old and fresh Atropine-Antacid Mixtures

△———△ Atropine solution

×– – –× Atropine-antacid mixture : fresh

⊙———⊙ Atropine-antacid mixture : old

(4) Assay of the Effects of Drugs on Experimental Peptic Ulcer

According to the method mentioned above, 30 rats were tested their susceptibility to ulceration and in 29 of them marked ulcers were found. In Table III, the results of another control group of 15 animals were shown. To express the degree of ulceration quantitatively, ulcers were classified into five groups according to their diameters, and the scores were given to each group as shown in Table III. Six animals out of 15 died from perforation in forestomach wall.

In groups treated with the drugs, the Atr. MeBr group had the score of 5.8±2.8, the D. P. B. group : 11.8±6.1, the Ben. MeBr group : 19.7±7.8, and control group 33.5± 8.5. Atr. MeBr showed marked preventive effects on ulcer formation, and only small ulcers were found, but there was no animal without ulceration. There were significant differences in the score between the control and the treated groups.

The dose of each drug was determined in order that they had nearly equal anticholinergic effects. The drugs were administered intraperitoneally three times at the interval of two hours. The doses of total amounts are shown in Table III. In Table IV

Table III. Effect of Some Anticholinergic Drugs on Gastric Ulcer Formation of Shay Rats

Drug	Dose (mg./kg.)	No. of animals	Number of Ulcers I	II	III	IV	V	Scors
Atropine methobromide	0.75	15	16.5± 5.9	1.5±1.0	0.31	0	0	5.8±2.8
D.P.B.[a]	7.5	15	43.1±13.1	3.0±1.47	1.85±100	0.5±0.6	0	11.8±6.1
Benactyzine methobromide	1.2	15	21.4± 8.7	4.8±2.3	1.4 ±1.06	0.5±0.5	0.5±0.7	19.7±7.8
Control		14	40.6±10.7	6.4±2.06	3.3 ±1.9	2.1±1.5	0.9±0.9	33.9±8.5

a) *d*-1,1-diphenyl-3-piperidinobutanol hydrochloride

Table of ulcer score

Group	Diameter of ulcer (mm.)	Score	Group	Diameter of ulces (mm.)	Score
I	0~2	0.2	IV	6~8	3.0
II	2~4	1.0	V	8	4.0
III	4~6	2.0			

Table IV. Effect of Some Anticholinergic Drugs on Gastric Secretion

Drug	No. of animals	Gastric juice ml./100 g. wt.	Gastric acid (mEq/1) Free acid	Total acid
Atropine methobromide	16	5.38±0.88	45.1±10.4	75.1±11.6
D.P.B.[a]	13	5.27±1.60	45.5±14.8	79.5±12.1
Benactyzine methobromide	15	6.33±0.83	38.7±15.2	68.1±14.1
Control	8	7.08±1.31	37.9±13.2	59.1±17.1

a) *d*-1,1-diphenyl-3-piperidinobutanol hydrochloride

the volume of gastric content and gastric acidity of each group was illustrated. The gastric contents involve the residue of food, gastric juice and saliva which was secreted in 19 hours after ligation of pylorus, but the greater part may be gastric juice. As to the volume of gastric contents, Atr. MeBr and D. P. B. group showed significantly lower value than control group, but the differences among them were smaller than that of the ulcer scores.

(5) Calculation of ED 50

According to the above method, the degree of ulceration can be expressed in score, but to make clearer comparison, the authors tried to use the incidence of ulcer as the all-or-none response.

Under the foregoing experimental conditions, ulcer formation was so heavy that for any drug it was difficult to inhibit the incidence of ulcer completely, The authors searched such a condition that the ulceration occurs in all treated animals without exception, even if moderately, and moreover it can be prevented with drugs perfectly. The following condition seemed to fulfill the above requirements; fasting periods: 48 hours, the time between operation and autopsy : 16 hours. Twenty control animals were used to test the incidence of ulcer under these conditions and all of them had moderate but distinct ulcer in their stomachs. Then we tested the effects of some drugs on this ulcer. The drugs to be tested were administered thrice a day during fasting periods and 3 times at the interval of 3 hours after operation. Preliminary dose during fasting periods was administered because these drugs were said to display higher effects clinically when they were administered successively for a few days. In Fig. 3 the dose-probit lines and ED 50 of every drug are shown. The dose means total dose except the preliminary doses. Eight animals were used for each dose.

In this experiment, the order of potencies of the tested drugs was; Atr. MeBr,> Ben. MeBr,>D. P. B., and synthetic aluminum silicate was found to be effective on this experimental ulcer. The effects of the drugs on gastric secretion for each dose were

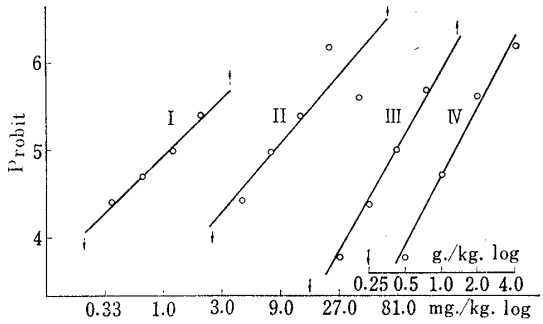

Fig. 3. Dose-probit Lines of Some Anticholinergic Drugs and an Antacid on Shay Rats

No.	Drug	ED_{50}	Fiducial limit (p=0.05)
I :	Atropine methobromide	1.30 mg./kg.	0.51～ 1.72 mg./kg.
II :	Benactyzine methobromide	9.12 mg./kg.	8.64～ 10.5 mg./kg.
III :	*dl*-1,1-Diphenyl-3-piperidinobutanol·HCl	86.52 mg./kg.	57.54～136.8 mg./kg.
IV :	Synthetic aluminum silicate	1.30 g./kg.	0.8 ～ 1.9 g./kg.

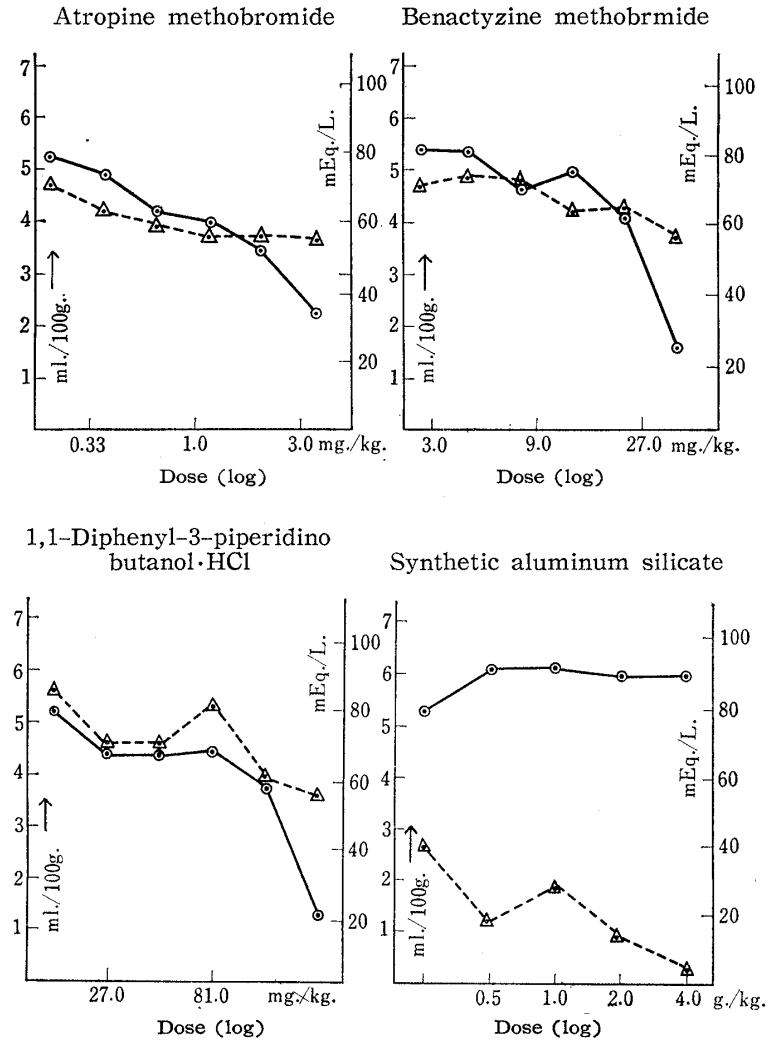

Fig. 4. Effects of Some Anticholinergic Drugs and an Antacid on Gastric Secretion and Gastric Acidity

△------△ Gastric juice volume ⊙——⊙ Free acid

illustrated in Fig. 4. Each curve has the tendency to decline slightly in proportion to dose but the variance of the responses seemed rather large.

(6) Effect of Combined Administration of Atropine Methobromide and Synthetic Aluminum Silicate

From the above results the authors believed that the ulceration of Shay rats could be treated all-or-none response under the foregoing conditions. With this method the benefits of combined administration of antispasmodics with antacid was examined. In this experiment, no preliminary dose was applied and 15 animals were used for each dose.

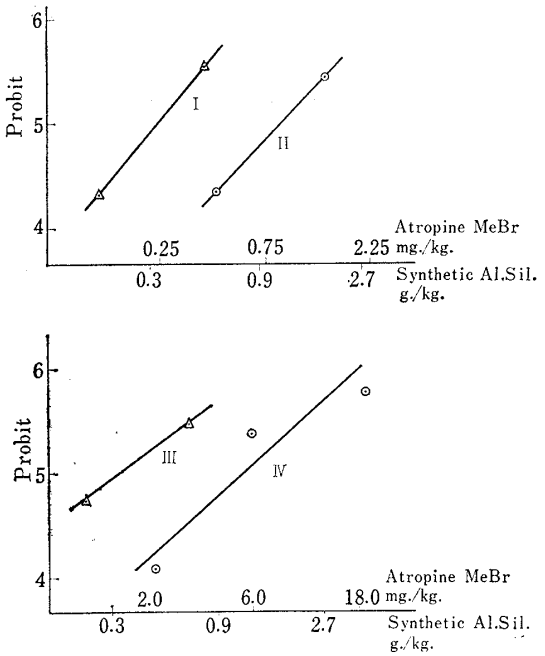

Fig. 5. Effect of Combined Administration of Atropine with Antacid on Shay Rats

I : Atropine methobromide (injection) + Synthetic aluminum silicate (oral)
II : Atropine methobromide (injection)
III : Atropine methobromide (oral) + Synthetic aluminum silicate (oral)
IV : Atropine methobromide (oral)

(15 animals for each dose)

As shown in Fig. 5, ED 50 of singly administered Atr. MeBr was 5.98 mg./kg. by oral route and 0.96 mg./kg. by intraperitoneal injection, and that of the synthetic aluminum silicate was 1.3 g./kg. by oral administration. ED 50 of the mixture which contained Atr. MeBr and aluminum silicate in the ratio of ED 50 of both drugs, in another words, the mixture of 5.98 mg. of Atr. MeBr with 1.3 g. of aluminum silicate, was calculated, and it corresponded to 1.47 mg./kg. of Atr. MeBr mixed in 0.31 g./kg. of synthetic aluminum silicate. They were equivalent to 1/4 of ED 50 of each drug. In the same way, ED 50 of combined administration of synthetic aluminum silicate by oral route and Atr. MeBr by injection was determined, and it corresponded to 0.3 g./kg. of aluminum silicate mixed with 0.24 mg./kg. of Atr. MeBr. This is also equivalent to 1/4 of ED 50 of each drug. From these results it may be shown that synthetic aluminum silicate potentiates the effect of Atr. MeBr to prevent gastric ulceration in rats.

Discussion and Conclusions

It is important to know the stability of synthetic antispasmodics mixed in antacids, for the mixture of both drugs is preferably prescribed to patients with peptic ulcer, but there are few reports about this problem. Grote, *et al.*[3] examined the stability of some synthetic antispasmodics mixed with aluminum antacids by calculating LD 90 of

3) I.V. Grote, M. Wood : J. Am. Pharm. Assoc., **47**, 785 (1958).

them, and concluded that antacids adsorbed much of the drugs but did not decompose them. From our results it has been shown that the antispasmodics we used are not decomposed even if they can not be detected by chemical analysis, and they are merely adsorbed by the antacid.

Elution of drugs from the mixture into artificial gastric juice was also examined and it was shown that the degree of elution was rather good but it was not the same among the drugs.

Combination of antispasmodics with antacid, however, is surely obstructing for the absorption of them from the gastrointestinal tracts, for the mydriatic action of atropine mixed with the antacid was smaller than pure atropine solution when they were administered orally. To find the convenient method to examine the therapeutic effects of drugs for peptic ulcer, Shay's method was employed and tried to compare the effects of a few drugs in doses which might have equal spasmolytic action on isolated small intestine of mouse. From these experiments the order of effects of these drugs was as follows; Atr. MeBr>Ben. MeBr>D. P. B. In this experiment the scoring system was employed to express the effects of the drugs. Keyriläniαn, *et al.*[4] described the distribution of points to express the severity of peptic ulceration in graded number as follows; to an ulcer with diameter of 0~2 mm.: 1 point was given, 2~5 mm.: 5 points, 5~10 mm.: 10 points, over 10 mm. : 20 points, and to perforation 20 points was given. The authors decided the distribution of scores on the basis of the observation that the occurrence of ulcers which were classified into 5 groups according to their diameters was inversely proportional to the scores. This distribution of scores resembles those of the Keyriläniαn's except that the perforation was not given the highest score. In case of perforation, we measured the diameter of the perforated ulcer and calculated the score as usual.

The order of effects inhibiting gastric acid secretion was; D. P. B.>Atr. MeBr>Ben. MeBr, but the difference between control group and drug treated groups was not so distinct as that of ulcer scores. As many authors described, the relation between inhibition of gastric secretion and suppression of ulcer formation was not strictly parallel. The three drugs seemed to have little difference in the dose of our experiments.

It seemed possible to regard the ulceration of Shay rat as the quantal response and to calculate ED 50 of drugs under the condition adopted by us. Both by scoring method and the probit method, approximately equal relative potencies were given to the three drugs. With the probit method the combined effect of antispasmodics and antacid was examined. By the combination of antispasmodics with antacid, preventive effect of ulcer formation of these drugs was potentiated, for 50% inhibition of the ulceration was attained by the combined dose of the 1/4 of the ED 50 of each drug.

The possibility of reducing the side effects of synthetic antispasmodics used for the therapy of peptic ulcer by the combination with antacids may be suggested from this result.

The authors heartily express our thanks for the support given to this research of the Grant for Researches by Minister of Education.

Summary

The stability of some antispasmodics mixed with antacid was examined, and the effect of combined administration of these two drugs was studied by experimental peptic ulcer. The antispasmodics used seemed not to be decomposed when they were mixed with synthetic aluminum silicate.

4) W. Keyriläniαn : Acta Pharmacol. Toxicol., **13**, 22 (1957).

From the resuls of the assay with mydriasis of mice eyes, is was shown that the antispasmodics by the antacid reduced the abesorption of these drugs from gastrointetinal tract, but practically this will not affect their pharmacological activity seriously, because gastric juice will facilitate the dissociation of the drug from antacid.

To examine the effects of the drugs on peptic ulcer, Shay rats were used. Ulcer formation on Shay rat's stomach was treated as the duantal response, and ED 50 of some drugs was calculated. By this method it was shown that synergism of antispasmodics with antacid was of the type of potentiation.

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UDC 547.496 : 548.2 : 543.422.4

207. Masao Nishikawa : Infrared Spectra of Thiourea and its Inclusion Compounds. IV.*1 Investigations on Aggregational States of Some Cyclohexane Derivatives.

(*Research Laboratories, Takeda Chemical Industries, Ltd.**2)

In an earlier paper of this series,[1] a method of investigations on infrared dichroism of thiourea adducts was described. This method would be also applicable for studies on hydrogen bonding of enclosed molecules having some polar groups.

Previously, Barlow and Corish[2] found that the C=O stretching vibrations of hexanoic acid and pelargonic acid molecules trapped in urea channels absorbed at the same frequencies as those in the liquid states and they suggested that the carboxyl groups of these molecules were in similar environments in these two states. An identical phenomenon was observed also by Mima, *et al.*[3] in the case of α-lipoic acid-thiourea adduct but more detailed studies did not appear to be published until the author measured the characteristic bands due to the OH out-of-plane bending vibrations of α-lipoic acid or hydnocarpic acid trapped in thiourea channels, and showed[1] that these carboxylic acids formed dimers in channels just as they did in nonpolar solvents.

As for the molecular structure, either of these acids has a relatively long CH_2 chain between the five-membered ring and carboxylic group and therefore, because of the flexibility of the polymethylene chain, the carboxyl groups will be able to form dimers without much difficulty even the molecules are confined in thiourea channels. If however, the flexible chain is missing or the carboxylic group is linked directly to the ring, hydrogen bonding of the acid dimers might not occur since the conformation of the ring is somewhat restricted as shown in the previous paper.*1 The present work is concerned with an analysis of aggregational states of cyclohexane derivatives having some polar groups directly attached to the six-membered ring. An outline of a part of this work was reported in a communication[4] already.

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*1 Part III : This Bulletin, **11**, 977 (1963).

*2 Juso-nishino-cho, Higashiyodogawa-ku, Osaka (西川正夫).

1) M. Nishikawa : This Bulletin, **10**, 1205 (1962).
2) G. B. Barlow, P. J. Corish : J. Chem. Soc., **1959**, 1706.
3) H. Mima, Y. Asahi, H. Okuto, T. Kanzawa : Yakugaku Zasshi, **80**, 1233 (1960).
4) M. Nishikawa : Chem. & Ind. (London.), **1963**, 256.