The authors are indebted to Takeda Chemical Industries Co., Ltd. for their kind supply of the starting materials for the present syntheses and to Dr. M. Sano of Central Research Laboratory, Daiichi Seiyaku Co., Ltd. for a gift of standard sample for identification. Thanks are also due to the Central Analysis Room of this Faculty for carring out the elemental analysis and optical measurements.

Summary

By condensation of 1-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)urea (Ⅳ)with malonyl chloride in pyridine, N,N′-bis(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosylcarbamoyl)-malondiamide (Ⅴ) was obtained. This compound, when treated with alkali, afforded each one mole of 1-(β-D-glucopyranosyl)urea and sodium 1-(β-D-glucopyranosyl)barbiturate (Ⅵ). Condensation of 1-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)urea (Ⅶ) with malonic acid in acetic anhydride gave N,N′-bis(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl-carbamoyl)malondiamide (Ⅷ) with a small amount of 1-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-5-acetylbarbituric acid (Ⅸ). On alkaline treatment, Ⅷ afforded sodium 1-(β-D-ribofuranosyl)barbiturate (Ⅹ) and 1-(β-D-ribofuranosyl)urea (Ⅺ). The overall yield of Ⅵ and Ⅹ from Ⅳ and Ⅶ were 30.3% and 37% respectively. The stucture of the glycosylbarbiturates, Ⅵ and Ⅹ, were discussed and their properties were described.

(Received November 5, 1963)

[Chem. Pharm. Bull. 12 (4) 465 ~ 472]

UDC 616.33-002.44-085

69. Keijiro Takagi, Yutaka Kasuya, and Kazuo Watanabe: Studies on the Drugs for Peptic Ulcer. A Reliable Method for Producing Stress Ulcer in Rats.

(*Department of Chemical Pharmacology, Faculty of Pharmaceutical Sciences, University of Tokyo*[*1])

For studying the remedies for peptic ulcer, we estimate their effectiveness on the experimental peptic ulcer of animals. In this field Shay's procedure[1] is the well established convenient method, but this is not enough for the research of complicated causative factors of peptic ulcer. Complexity of the etiology of gastric ulcer made many researchers to find a variety of experimental procedures to produce this lesion on animals.[1~11]

---

*1 Hongo, Tokyo (高木敬次郎, 粕谷 豊, 渡辺和夫).

1) H. Shay, S. A. Komarov, S. S. Fels, D. Meranze, H. Siplet: Gastroenterology, **5**, 43 (1945).
2) H. Selye: Nature, **138**, 32 (1936).
3) A. Robert, J. E. Nezamis: Proc. Soc. Exp. Biol. Med., **99**, 443 (1958).
4) M. Levrat, R. Lambert: Gastroenterology, **37**, 421 (1959).
5) J. Watt, C. W. M. Wilson: *Ibid.*, **37**, 8 (1959).
6) S. Bonfils, G. Rossi. G. Liefooghe, A. Lambling: Rev. Franc. Clin. Biol., **4**, 888 (1959).
7) J. D. Dahl, R. K. Blaisdell, E. Beutler: Proc. Soc. Exp. Biol. Med., **101**, 622 (1959).
8) C. R. Thomas, C. L. Dorolle, C. R. Effron, G. Volunter, M. Meyer, J. M. Chaumontet, D. Larue: Arzneim. Forsch., **10**, 589 (1960).
9) H. Selye, P. Jean, M. Cantin: Proc. Soc. Exp. Biol. Med., **103**, 444 (1960).
10) D. A. Brodie: Gastroenterology, **43**, 107 (1962).
11) T. Toriumi: Tokyo Jikeikai Med. J., **71**, 1876 (1956).

Of these procedures, so called "stress ulcer" seems competent to be used for the assay of the effects of drugs for peptic ulcer. Because, as clarified in this report, this ulcer has many convenient and unique properties, we tried to determine the experimental conditions for steady production of this ulcer in rats. Under these conditions, we examined the effects of some drugs, particularly that of anticholinergics, tranquilizers, and of their combination.

Methods

Rats weighing 150~180 g. were used. After 24 hrs.' fasting, rats were fixed on their backs by the limbs on boards and immersed to the depth of the xiphoid in a water bath whose temperature was kept at 25°. The rats were autopsied after 20 hrs.' stress. Their stomachs were excised and incisions were made along the greater curvature. The stomach walls were observed carefully by naked eyes or by magnifying glass if necessary. pH of the gastric mucosa was measured with paper indicator of Toyo Filter Paper Co., Ltd. soon after sacrifice. After these observations, stomachs were immersed in 20% formaline solution to prepare the preparation for the histological examination.

The drugs were usually injected subcutaneously 10 min. before the application of the stress. Other routes of administration were employed when they were necessary.

Results

The Incidence of Stress Ulcer

Fifty animals were stressed and ulcers were found in 49 of their stomachs. Then the incidence of this stress ulcer is 98%. As many authors described, the ulcers occurred in the glandular portion of the stomach and the forestomachs remained almost intact. The samples of this ulceration were shown in Fig. 1, and microscopic pictures of the section were shown in Fig. 2. The total number of 236 ulcers occurred in 49 rats were distributed in glandular portion as illustrated in Fig. 3. The highest frequency of ulcer occurrence was observed in the portion (Ⅱ), in which there are much of the gathers of mucosa, along which the ulcers were found as long or round lesions with obscure edge. In the histological examination, none of these lesions perforated or eroded muscularis mucosae, so that these lesions can not be said as gastric ulcer in a strict sense. But many authors[10] call this lesion "stress ulcer," then we, too, follow them. Through observation with naked eyes the severity of ulcers for each animal was graded in four groups; (−) : the ulcer was not found, (+) : one or a few ulcers were found but they were not remarkable, (++) : a few ulcers which were moderately severe in size or depth, (+++) : a few of very severe ulcers or a number of moderate ulcers were found. In Fig. 4 the severity of ulceration and the number of the ulcer in each of 49 animals were illustrated. This shows that the ulcer formation of two thirds of stressed rats were very remarkable and the mean number of ulcers per animal was about 5.

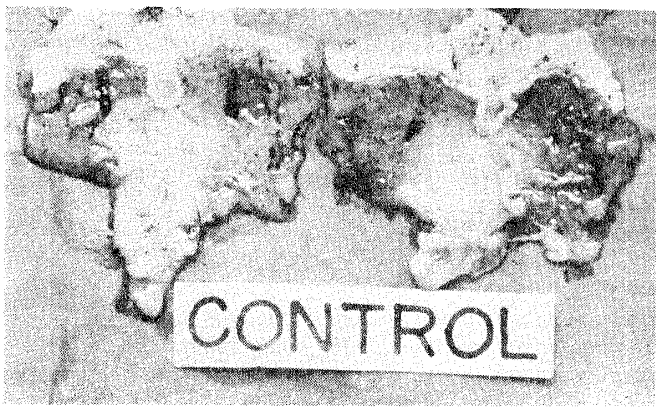


Fig. 1. Photograph of Inner Surface of Rat's Stomach showing the Lesions caused by Restraint and Water immersing Stress

Such distinctiveness and constancy of the response may suggest the availability of this ulcer for the estimation of the effects of antiulcer drugs. To make this possibility clearer, we examined some characteristics of this ulceration and compared this with other kinds of stress ulcers.

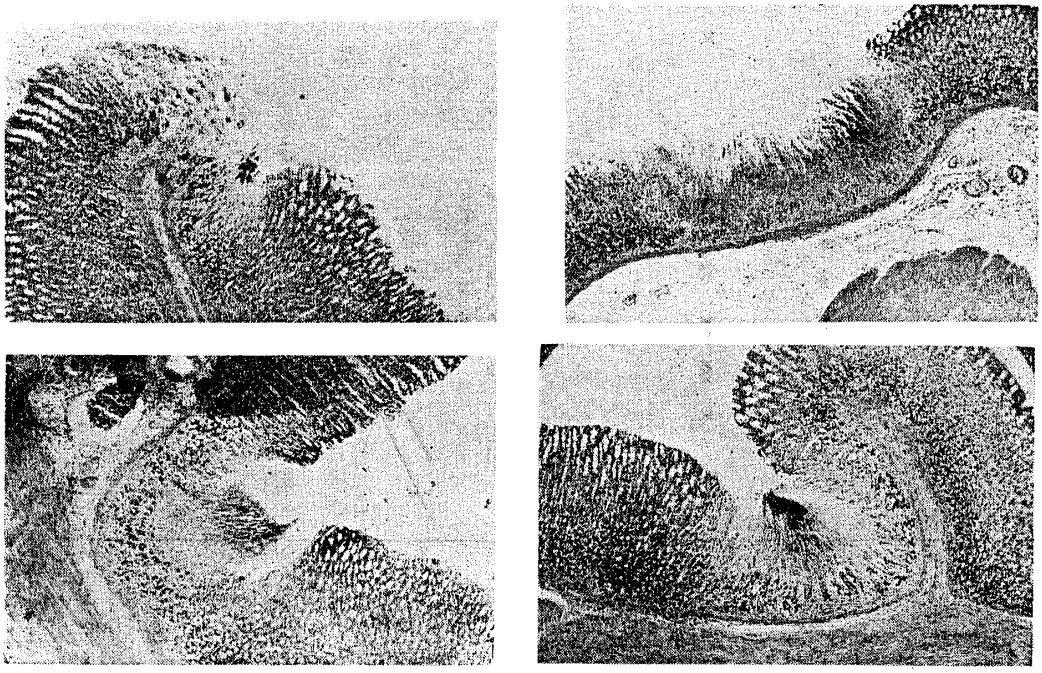
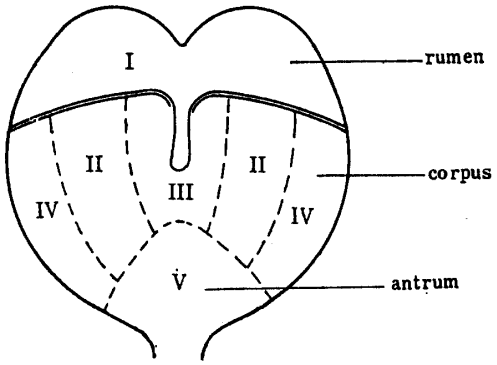


Fig. 2. Microscopic Photograph of the Lesions caused by Restraint and Water immersing Stress



Portion	No. of ulcers	%
I	0	0
II	167	66
III	17	8
IV	52	22
V	8	4

Fig. 3. Distribution of Ulcers

Time Necessary for Development of the Ulcer

As illustrated in Fig. 5, the ulcers occurred in most of the stressed animals in 15 or 20 hours and the maximal response was observed in 20 hours. Over these time, further development of ulcer did not occur, but the motality rather increased. Then the optimal time of stress duration for the uniform production of ulcer may be 20 hours.

Temperature of Water Bath

In water of 20°, some of the rats treated with hypothermic drugs like chlorpromazine or urethane died.

In water over 30°, some rats tolerated the stress without any affections in their stomachs. Then 25° may be the most convenient temperature for uniform production of ulcer. In water of this temperature, we obtained the results described in Fig. 3.

Difference of Response between Sex

Difference of the response to stress between both sexes was examined. The results were shown in Table I. In general, female animal is said to be less sensitive to stress.

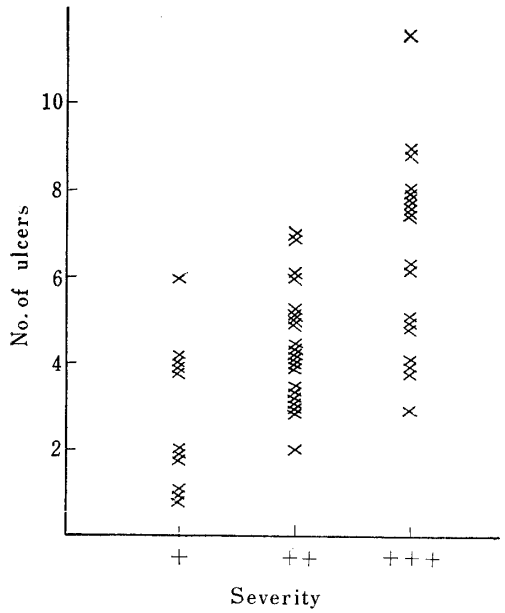


Fig. 4. Relation between Approximate Classification of Severity of Ulceration and Number of Ulcers for each Animal

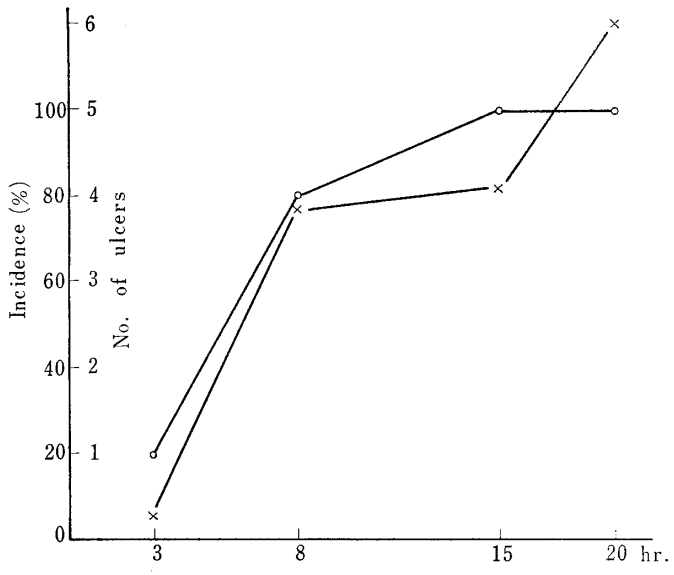


Fig. 5. Relation between Stress Duration and Development of Stress Ulcer

—O— incidence %
—×— no. of ulcers per animal
5 animals for each time

Table I. Differences of Susceptibility between Sexes

Sex	Number of animals	Ulcer						Hemorrhage incidence
		Incidence (%)	Number of ulcer	Severity				
				##	#	+	−	(%)
Male	10	100	5.5	5	2	3	0	100
Female	10	100	4.9	6	3	1	0	100

but in our experiment both sexes did not show any significant difference in their response to stress.

Comparison of Various Stressors

The stress ulcer have been induced by various kinds of stressors.[10] Most of these stressors were found in course of the research on the etiology of peptic ulcer.

Then many authors were not interested in the incidence rate or the availability to pharmacological study of experimental ulcer. With regard to these view points we compared the water immersing stress with other stressors which were used by some authors.

Restraint stress was given to rats by fixing them on boards in dorsal position and keeping this pose for 24 hours. In electroshock stress, rats were immobilized by fixation on boards and electroshock of 40 to 50 volt was charged between hind limbs in an interval of 10 seconds in a duration of about 0.5 second for 24 hours. In the case of the stress of low temperature, rats were fixed on boards and kept in the room of about 7° for 24 hours. The stress box method was used by Toriumi[11] to examine the role of emotional stress in the cause of peptic ulcer. The stress box is equipped with three stressors, the bothers for noise, the electric bulb for flashing and the floor of metal bars for electrical stimulation. These three work in turn in an interval of 10 seconds in a duration of 0.5 second. Rats were kept in this box for 24 hours after

the fasting period of 24 hours. The results of the comparison of the effects of these stressors were summarized in Table Ⅱ. From the view point of bioassay, the important characteristics are the incidence of ulcer formation and the mortality. By the stress of low temperature and electroshock more animals died accidentally earlier than 24 hours. Moreover, inspite of such severity of stress, some of the stomachs of stressed animals ramained unaltered.

Of these stressors, water immersing stress seemed to have displayed most desirable charactors for bioassay, namely low mortality and high rate of incidence of ulceration.

Table Ⅱ. Comparision of some Stressors

	Restraint	Electroshock	Cold	Stress box	Immerse in water
Incidence (%)	66	50	60	63	98
No. of animals	24	20	10	30	50
No. of ulcers	2.0	2.8	2.6	2.0	5.5
Mortality (%)	0	25	100	23	0

Sensitivity of Ulceration to Drug Treatment

Five different doses of atropine methobromide were administered to animals to examine the dose-response relations in preventive effects of ulceration. The dose of 5 mg./kg. of the drug markedly prevented the occurrence of the ulcer, and in accordance with the increase of the dose the prevention became more perfect and 25 mg./kg. of the drug completely prevented the ulceration in 10 animals. These results were illustrated in Fig. 6.

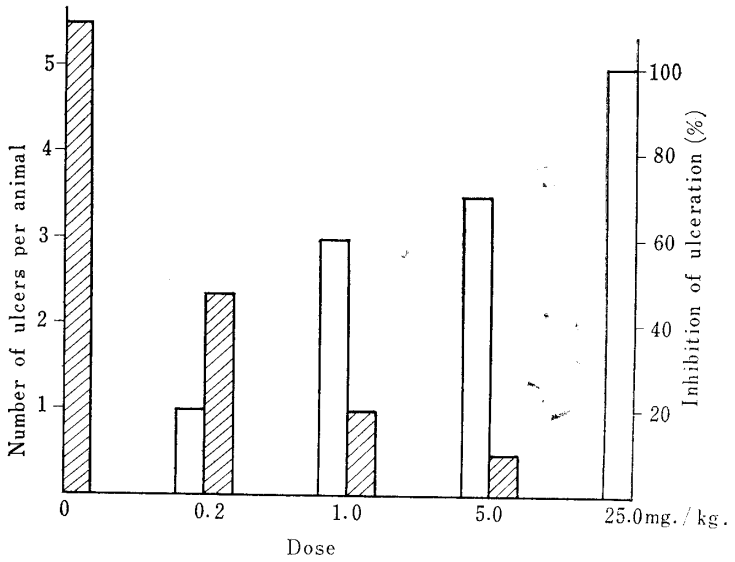


Fig. 6. Effect of Atropine Methobromide on Stress Ulcer

We examined some other anticholinergic drugs and studied the mode of action of them. These results will be reported elsewhere.

Effects of some Drugs other than Anticholinergics on Stress Ulcer

a) Sedatives and tranquilizers : Effects of chlorpromazine, meprobamate and bromvalerylurea were examined. Two drugs other than chlorpromazine are proved to have little effects on peripheral autonomic nervous system and on Shay ulcer in limited dose, so we had expected the possibility that some parts of the role of the central narvous system in occurrence of stress ulcer would be proved by this experiment.

Chlorpromazine had remarkable inhibitory effects in a dose of 10 or 20 mg./kg. In this dose, the main symptoms of animals were ataxia and hypothermia. In spite of marked hypothermia the animals tolerated well the stress and none of them died, while in case of urethane or barbiturates the mortality was very high. Meprobamate showed distinct effect when the high dose of 200 or 300 mg./kg. was administered, but even by

the highest dose the complete suppression of ulcer formation was not attained. Bromvalerylurea scarcely inhibited the stress ulcer. These results are shown in Table Ⅲ.

b) Miscellaneous drugs : Sodium phenobarbital, caffeine, chlorpheniramine maleate, hydrocortisone and papaverine were tested.

Phenobarbital was not effective to prevent the ulceration. By the dose of 100 mg./kg., incidence of ulcer and hemorrhage was 100%. Over this dose animals died as the result of lowering of body temperature. Caffeine was expected to make the ulcer more remarkable by its central nervous system stimulative action and secretagogue action. But by the dose of 40 mg./kg. any significant alteration was not shown in severity of ulcer compared with control animals.

Hydrocortisone is used for the production of experimental peptic ulcer in rats in some reports.[7~9]

But a dose of 25 mg./kg. of hydrocortisone did not display any effects on stress ulcer. Papaverine had slightly prevented ulceration in the dose of 75 mg./kg. but increasing the dose the complete inhibition was not attained. These results are summarized in Table Ⅳ.

Table Ⅲ. Effects of Central Nervous System Depressants on Stress Ulcer

Drugs	Dose (i.p.) (mg./kg.)	Ulcers		Hemorrhage incidence (%)
		Incidence (%)	No. of ulcers	
Chlorpromazine	10	40	1.6	50
	20	20	0.5	20
Meprobamate	100	80	3.0	80
	200	60	2.4	80
	300	50	2.7	50
Bromvalerylurea	250	90	3.1	90
Control	—	100	5.0	100

Ten animals were used for each treatment.

Table Ⅳ. Effects of Miscellaneous Drugs on Stress Ulcer

Drugs	Dose (i.p.) (mg./kg.)	Ulcers		Hemorrhage incidence (%)
		Incidence[a] (%)	No. of ulces animal	
Phenobarbital sodium	40	100	4.0	100
Caffeine	40	100	6.0	100
Chlorpheniramine maleate	100	50	1.7	40
Hydrocortisone	25 (s.c.)	90	5.0	90
Papaverine hydrochloride	75	70	4.2	80
Control	—	100	4.6	100

a) Ten animals were used for each treatment.

Effects of Combined Administration of Anticholinergic Drugs and Central Nervous System Depressive Drugs

From the view of the therapy of peptic ulcer, combination of a peripherally acting drug and a centrally acting drug seems to be favourable. Some of these drugs were proved to be effective by themselves in preventing stress ulcer by the above experiments. Then we planned to examine the efficacy of the combination of these drugs on experimental animals.

First, the approximate minimal effective dose of each drug was determined by preliminary experiments, and then two of them to be combined were administered simultaneously to the rats to be stressed. The degree of suppression of ulcer formation by the combined drugs was compared with that of each drug. In Table V, the mbined effect of 1.0 or 0.2 mg./kg. atropine methobromide with 100 mg./kg. of meprobamate was shown. The incidence of ulceration was remarkably lowered by this combination. In the same procedure, beneficial effects of combination were examined in other pairs of drugs. In each combination marked potentiation was observed in the suppressive effect of ulceration in rats. These results are summarized in Table VI.

Table V. Combined Effect of Meprobamate and Atropine Methobromide

Treatment	Dose (mg./kg.)	Incidence of ulcer (%)	Severity			
			##	#	+	−
Atropine methobromide	1.0 (s.c.)	40	0	1	3	6
	0.2 (〃)	80	2	2	4	2
Meprobamate	100 (i.p.)	80	2	5	1	2
Atropine methobromide + meprobamate	1.0 (s.c.) 100 (i.p.)	0	0	0	0	10
Atropine methobromide + meprobamate	0.2 (s.c.) 100 (i.p.)	30	0	0	3	7
Control		100	4	5	1	0

Ten animals were used for each treatment.

Table VI. Combined Effects of some Central Nervous System Depressants and Anticholinergics on Stress Ulcer

Drugs	Dose (mg./kg.)	Incidence of Ulcer, % in 10 Animals		
		Single administration	Combination	
Control	—	100		
Atropine methobromide	1.0 (s.c.)	40		
Bromvalerylurea	250 (p.o.)	80	with atropine methobromide 1.0 mg./kg.	20
Thalidomide	400 (〃)	70	〃	0
Clidinium	0.25 (s.p.)	90		
Chlordiazepoxide	100 (〃)	80	with clidinium 0.25 mg./kg.	40
Isopropamide	1.0 (〃)	60		
Emilcamate	200 (p.o.)	100	with isopropamide 1.0 mg./kg.	40

Discussion

There are plenty of the reports to produce peptic ulcer on experimental animals. Because none of these methods have elucidated its true etiology, there will be many risks or errors in using experimental ulcer for screening of new drugs or for the analysis of etiology of the disease of man. However, with due regard to these points, there is urgent request for these experimental methods, because it is not enough to assay the effects of peptic ulcer remedies only with the inhibition of gastric acid secretion or with spasmolytic activities, since many causative factors were detected by recent studies.

Stress ulcer may, we think, fulfill some parts of the requirements. Simplicity of experimental condition without any agents or any complicated surgical treatments is convenient for screening test, and experiments under comparatively physiological condition will promise the possibility that the etiology of this ulcer has some analogies to

that of man in many respects. But strictly speaking, there are some doubts concerning to this analogy. We keep ourselves from this problem until the decisive answer will be given to it. Our study is aimed, for the present, at the uniform production of stress ulcer regardless to its etiology. In this respect, some successes were obtained owing to introduction of the stress to fix and immerse the rat in water. When this stress was compared with other kinds of stress, it gave the highest incidence of ulcer and lowest mortality. Sensitivity of this ulcer to drugs, which were known to have some effects on the disease, was examined. We were interested especially in the effects of anticholinergics and tranquilizers because they are the main remedies for the disease in recent years. Using atropine methobromide we have shown the linear relationship between the dose of the drug and its inhibitory activity of ulcer formation. Only one dose of 1 mg./kg. of the drug was markedly effective in suppressing the ulcer formation. This suggests that parasympathetic nervous system may play an important role in stress ulcer formation. Effects of two tranquilizers, chlorpromazine and meprobamate on stress ulcer were also examined.

Both drugs significantly inhibited the ulceration, but the latter seemed somewhat incomplete. These results may suggest the participation of the central nervous system to the etiology of the stress ulcer. In the preceeding reports, we proved that the preventive effect of anticholinergics could be potentiated by the simultaneous administration of an antacid.

The synergism between anticholinergics and central depressants has been expected and utilized in clinical practice.

Combined administration of anticholinergics and central nervous system depressants was proved to have stronger inhibitory activity on stress ulcer than the single administration of each drug by our experiments. As well as the tranquilizers, sedatives as bromvalerylurea or thalidomide showed the same synergistic effects with anticholinergics.

Summary and Conclusions

A simple and reliable method for production of gastric ulcer on rats by stress was described.

Rats fixed on boards were immersed in water of 25° to the depth of xiphoide. After 20 hours, marked gastric erosions were found in the glandular portion of the stomach. This stress is more favourable than other stresses as cold, restraint, or emotional stress by stress box in view of incidence and mortality.

Anticholinergics or central depressants effectively inhibited the ulceration and combination of both drugs suppressed it more effectively than single administration of each drug.

(Received December 6, 1963)