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Inhibitory Effects of Histamine Receptor Antagonist on Stress Ulcer*)

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ABSTRACT

Cold restraint ulcers were produced in rats for use as models for stress ulcers, and the inhibitory effects of histamine H_1- and H_2-receptor antagonists on ulcerogenesis were studied from the view points of gastric acidity, intra-gastric mucosal content of histamine and microvasculature of the gastric mucosa. The following results were obtained.

1. Both diphenhydramine, an H_1-antagonist, and cimetidine, an H_2-antagonist, successfully inhibited development of cold restraint ulcers.
2. Study of gastric acidity at time of cold restraint ulcer formation failed to demonstrate a state of hyperchlorhydria in the control group, diphenhydramine group and cimetidine group.
3. The microvasculature under cold restraint showed disturbance of microcirculation in the gastric mucosa due to stasis of blood in the control group, but blood flow was good in both the diphenhydramine and cimetidine administered groups.
4. The histamine content in the gastric mucosa under cold restraint showed a rapid change in pattern in the control group from a significant decrease 30 minutes after cold restraint to a significant increase at 120 minutes. The diphenhydramine administered group presented a histamine release inhibitory pattern while the cimetidine administered group showed a change in pattern similar to the controls.

From the above results, it is noted that the histamine released from the gastric mucosa under cold restraint acts upon the microvasculature rather than gastric acid secretion, and induces disturbance of the microcirculation causing devitalization of the gastric mucosa due to hypoxia, and thus contributes to ulcerogenesis. At this time, it is considered that diphenhydramine with its powerful sedative effect on the brain reduces the brain reaction to stress and secondarily inhibits release of histamine, while cimetidine blocks, at the histamine H_2-receptor site, the vascular effects of histamine released under cold restraint and prevents the development of microcirculatory disturbance. Therefore, when the development of acute gastro-duodenal mucosal lesions (AGML) can be anticipated clinically, it is felt the administration of histamine receptor antagonist can provide some degree of preventive action towards AGML.

INTRODUCTION

Acute gastro-duodenal mucosal lesions (AGML) is an entity which presents the initial symptoms of hematemesis and melena, and it is frequently difficult to arrest bleeding, thus

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resulting in death. The developmental mechanism of this disease which is treated as a stress ulcer has heen studied from the ulcerogenic mechanism proposed by Shay and Sun[18], but there is no concensus as to which are more important the aggressive factor or the mucosal defensive factors. There is a large amount of histamine in the gastric mucosa, and it is assumed that it has such pharmacological actions which induced hypersecretion of gastric acid and vascular action, and is also strongly involved in both aggressive and defensive factors. Therefore, in this experiment the changes in histamine content in gastric mucosa under stress, gastric acidity as an aggressive factor and mucosal resistance as a mucosal defensive factor were studied. Also study was made on the AGML inhibitory effects of histamine H_1- and H_2-receptor antagonists.

METHOD OF EXPERIMENT

1. Experimental animals

The experimental animals used were Wistar strain male rats weighing 200-250 g. A metal cannula (outer diameter 0.4 cm, inner diameter 0.3 cm, length 2.0 cm) were inserted into the fore stomach and antrum[2], and the rats were bred for about 2 months. On the day prior to the experiment, the stomach was washed with saline to eliminate food residue, and then they were fasted for 24 hours. The rats were divided into the following 3 groups.

Control group: Only gastrostomy was performed.

Diphenhydramine injected group: Diphenhydramine (10 mg/kg), an H_1-antagonist, was injected intramuscularly 30 minutes befor cold reatrains.

Cimetidine injected group: Cimetidine (48 mg/kg), an H_2-antagonist, was injected intravenously into the tail vein.

2. Method of cold restraint

The cannula of the gastric fistula was fitted with an inner cylinder to prevent leakage of gastric fluid, and the rats were placed in wire restraint cages in accordance with the Senay method and exposed to cold in a room maintained at 4°C[17].

3. Observation of ulcers

Gastric fluid of the rats was collected to determine gastric acidity, 30, 60 and 120 minutes after cold restraint and decapitated, after which the stomach was excised. Check was made for hemorrhage and erosion using a stereo-microscope.

4. Determination of gastric acidity

The gastric acidity of non-restraint rats in each group was considered the value of basic acid secretion, and the stomach of restraint rats was washed with 10 ml of pH 8.5 NaOH solution at 30, 60 and 120 minutes after cold restraint by a cannula inserted through the fore stomach. The gastric fluid was collected and with an autburette titrated up to pH 7.0 using 0.005N NaOH solution after which the total acid was determined.

5. Method for observation of gastric mucosal microvasculature and RBC distribution within capillaries

Upon completion of cold restraint, 1.0 ml of FITC-dextran 10% saline with a mean molecular weight of 40,000 was slowly injected into the tail vein without anesthesia, and the stomach was excised after confirming by the check of the yellow staining of the conjunctiva that the dextran was circulating throughout the body uniformly. The excised stomach was fixed in 20% formalin for 5-6 hours, and the fundus of the stomach was cut into 2-3 mm sections. Paraffin blocks were prepared and the specimens were sliced into thin sections of 30 μ. The

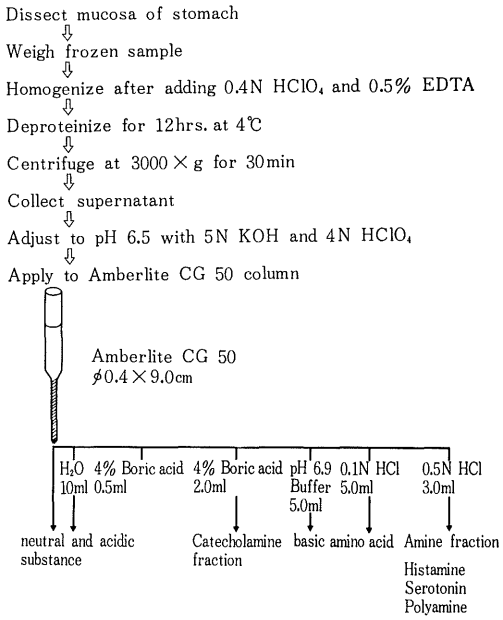

Fig. 1. Tissue histamine extraction method according to Wada et al.

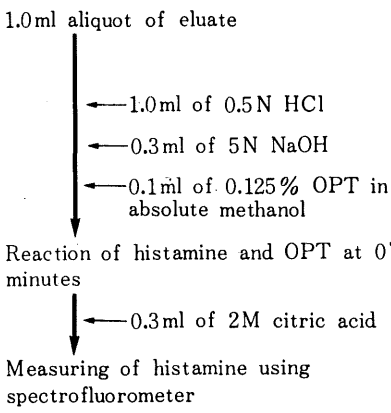

Fig. 2. The method for the fluorometric assay of histamine. (a modification of Shore's method)

microvasculature was observed under a fluorescent microscope. The distribution of RBC was observed by H-E staining of 3 μ slices taken from areas adjacent to the site from where sections had been removed for observation of the microvasculature.

6. Determination of intramucosal histamine content

The intramucosal histamine content of rats not subjected to cold restraint in each groups was used as the pre-test value. The stomach was excised 30, 60 and 120 minutes after cold restraint, and the mucosa of the gastric fundus was detached and collected. Histamine was extracted by the method of Wada et al.[21] using a column with Amberlite CG 50 resin, and determination was made by a modified method of Shore[19] (Fig. 1, 2).

RESULTS OF EXPERIMENT

1. Incidence of ulcers

The incidence of ulcers in the controls was 2 out of 8 (25%) in the 30-minute cold restraint rats, 7 out of 8 (88%) in the 60-minute restraint rats and 7 out of 8 (88%) in the 120-minute restraint rats, indicating a sharp increase after 60 minutes of cold restraint. The incidence of ulcers in the diphenhydramine injected group was 3 out of 12 (25%) in the 30-minute restraint rats, 5 out of 12 (41%) in the 60-minute restraint rats and 10 out of 12 (83%) in the 120-minute restraint rats. Although there was about 50% inhibition in the 60-minute restraint rats of the contlors, in those after 120-minutes of cold restraint the incidence was about the same as the control group. In the cimetidine injected group, no ulcers were noted in the 30-minute cold restraint rats, but were noted in 4 out of 12 (33%) in the 60-minute restraint rats and 7 out of 12 (58%) in the 120-minute restraint rats, indicating that they were markedly inhibited as compared against the controls (Table 1). Hemorrhage and erosion were limited to the gastric fundus in all groups and none were observed in the antrum or duodenum (Fig. 3).

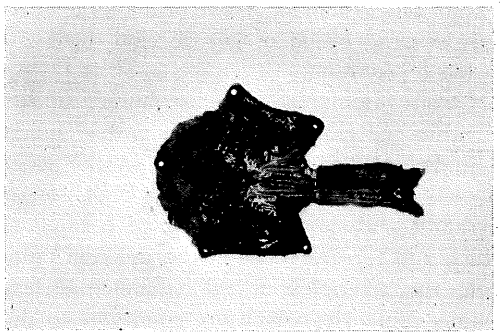

Fig. 3. Macroscopic finging of cold resraint ulcers

2. Changes in gastric acidity

The basal gastric acid secretion of the control group was 3.0 ± 0.5 μEq/ml (M$\pm$SD, n=4), and was 1.3 ± 0.5 μEq/ml (M$\pm$SD, n=4) in

Table 1. Incidence of gastric hemorrhage and erosion after cold restraint

cold restraint (min)	30′		60′		120′	
Group	n^a/n^b	%	n^a/n^b	%	n^a/n^b	%
Control	2/8	25	7/8	88	7/8	88
Diphenhydramine administered	3/12	25	5/12	41	10/12	83
Cimetidine administered	0/12	0	4/12	33	7/12	58

n^a=number of rats with lesion
n^b=number of rats

the 30-minute restraint rats, 1.6±0.3 μEq/ml (M±SD, n=4) in the 60-minute restraint rats and 3.4±0.8 μEq/ml (M±SD, n=4) in the 120-minute restraint rats. The gastric acidity of the 30-minute and 60-minute restraint rats were significantly decreased ($p<0.01$, $p<0.01$ respectively) as compared to the basel level of gastric acid secretion, while that of the 120-minute rats failed to demonstrate a significant difference (N. S.). Thus, the gastric acidity during cold restraint was not accelerated. The gastric acidity of the diphenhydramine injected group showed a basal gastric secretion of 3.0±1.5 μEq/ml (M±SD, n=4), and was 1.9±1.0 μEq/ml (M±SD, n=4) in the 30-minute restraint rats, 2.0±0.8 μEq/ml (M±SD, n=4) in the 120-minute restraint rats, while the acidity in the cimetidine injected group indicated a basal gastric secretion of 1.1±0.6 μEq/ml (M±SD, n=4), and was 0.7±0.4 μEq/ml (M±SD, n=4) in the 30-minute rats, 0.7±0.4 μEq/ml (M±SD, n=4) in the 60-minute rats and 3.0±0.7 μEq/ml (M±SD, n=4) in the 120-minute rats (Table 2). None of the three groups showed acceleration of gastric acid during the period of restraint.

3. Microvasculature and distribution of RBC within the capillaries of the gastric mucosa

Findings of microvasculature in the gastric mucosa of rats not treated by the FITC-dextran method are as shown in Fig. 4a. i. e. capillaries which run tortuosly within the mucosal branch from the arterioles of the submucosal layer, venous capillaries which are connected to these capillaries and line the superficial layer horizontally like golden lace, and collecting venules which descend almost vertically from the superficial layer to within the mucosa are all clearly visualized by FITC-dextran fluorescence, while only a small number of RBC could be observed within the venous capillaries by H-E stain (Fig. 4b).

Only slight FITC-dextran fluorescence could be observed in the venous capillaries of the mucosa in the 60-minute restraint rats of the control group, and the collecting venules and the capillaries could not be visualized, demonstrating findings indicative of circulatory disturbance. The RBC distribution within the mucosal capillaries showed many RBC in the venous capillaries and collecting venules, and in some areas there was rouleau formation, presenting findings of stasis of blood (Fig. 5a, 5b). The findings of the 60-minute restraint rats in the diphenhydramine injected group showed the venous capillaries and collecting venules to be clearly visualized, a small number of RBC were observed in the venous capillaries and the mucosal microvasculature was well preserved (Fig. 6a, 6b), and in the 120-minute rats findings of disturbance in microcirculation due to blood stasis were noted (Fig. 7a, 7b). The capillaries, venous capillaries and collecting venules were well visualized in the 60-minute restraint rats of the cimetidine injected group, and the mucosal microcirculation was well preserved (Fig. 8a, 8b).

4. Histamine content in gastric mucosa

The pre-test gastric mucosal histamine content of the controls 8.4±1.1 μg/g (M±SD, n=6), while after 30, 60, 120 minutes of cold restraint, it was 4.1±1.5, 8.3±1.2 and 11.9±2.2 μg/g. A significant decrease ($p<0.01$) was noted at 30 minutes after which it increased. The pre-test value for diphenhydramine group was 7.3±0.6 μg/g (M±SD, n=6), while after restraint for 30, 60 and 120 minutes, the values were 13.3±2.8, 12.5±1.5 and 10.6±2.4 μg/g respectively. A significant increase ($p<0.01$) wa noted at 30 and 60 minutes reatraint rats. The

Table 2. Gastric acid concentration after cold restraint

		acid concentration (μEq/ml)			
cold restraint (min)		0′	30′	60′	120′
Control	(n=4)	3.0±0.5	1.2±0.3*	1.6±0.3*	3.4±0.8
Diphenhydramine administered	(n=4)	3.0±1.5	1.9±1.0	2.0±0.8	2.0±1.0
Cimetidine administered	(n=4)	1.1±0.6	0.7±0.6*	0.7±0.4*	3.1±0.7

* $p<0.01$ (M±SD)

* Significance of the difference between means of the pre-test value and restrained value within each study group.

Table 3. Gastric mucosal histamine content after cold restraint

		mucosal histamine content (μg/g)			
cold restraint (min)		0′	30′	60′	120′
Control	(n=6)	8.4±1.1	4.1±1.5*	8.3±1.2	11.9±2.2*
Diphenhydramine administered	(n=6)	7.3±0.6	13.3±2.8*	12.5±1.5*	10.6±2.4
Cimetidine administered	(n=6)	13.3±2.3	9.1±1.6*	8.8±2.3*	9.6±2.0

* $p<0.01$ (M±SD)

* Significance of the difference between means of the pre-test value and restrained value within each study group.

pre-test value for cimetidine group was 13.3± 2.3 μg/g (M±SD, n=6), while after restraint for 30, 60 and 120 minutes, the values were 9.1±1.6, 8.8±2.3 and 9.6±2.0 μg/g respectively, with the values up to 60 minutes being significantly decreased ($p<0.01$) (Table 3).

DISCUSSION

First, review will be made of gastric acid which is said to be an aggressive factor in ulserogenesis. There are contradicting reports on changes in gastric acidity in experimental models under stress, i.e. some claim it increases[3,9] while others report it decreases[4,12]. Our findings on ulcerogenesis in restraint rats and changes in gastric acidity show that there was a marked increase in incidence of ulcerogenesis in the 60-minute restraint rats of the control group, while the gastric acidity was significantly decreased as compared to the basal acid secretion leading us to surmise that increase in gastric acidity is not the primary consideration in the formation of cold restraint ulcers, but that the involvement of mucosal defensive factors is more important.

Of the various mucosal defensive factors, microcirculation which has effects upon mucosal resistance was first advocated by Virchow[20] in 1853, and has been studied subsequently by many workers[6,8]. In summary, it is reported that disturbance of microcirculation is due to the two hemodynamic states of 1) decrease in the gastric mucosal circulation caused by contracture of arterioles and 2) states of mucosal blood flow, plus the combined effects of the two. This circulatory disturbance causes hypoxia of the gastric mucosa and results in its devitalization. The authors' results on the relationship between development of cold restraint ulcers and mucosal microcirculation showed that the microvasculature of the 60-minute restraint rats of the control group in which ulsers were noted in 88%, presented findings of disturbance in mucosal microcirculation and H-E stain showed findings of blood stasis. These findings make us presume hypoxia of the gastric mucosa caused by stasis of blood flow is an important factor in the development of restraint ulcers.

There are conflicting reports on the changes in histamine content in the gastric micosa during stress, some claiming there is no change[10] and others report increases[13] while still others have noted decreases[7]. The determinations made by the authors over time under the state of cold restraint showed that mucosal histamine content decreased significantly in the 30-minute rats and adruptly increased significantly in the 120-minute rats. Thus, it is presumed that stress releases intramucosal histamine and also promotes its synthesis.

From the above findings, it was concluded that disturbance of mucosal microcirculation is more important in the development of cold restraint ulcers than acceleration of gastric acid secretion, and that stasis of blood caused by vascular action due to histamine released from the gastric mucosa under cold restraint is involved in the disturbance of microcirculation.

Next, consideration will be given to the restraint ulcer inhibiting effect of histamine receptor antagonists. First, the effect of histamine receptor antagonists on histamine induced acceleration of gastric acid secretion was reviewed. There is a report by Loew et al.[11] on the inhibitory effect of H_1-receptor antagonists on gastric acid secretion, but at present there is a negative attitude towards this report. On the other hand, there are many reports including

the one by Black et al.[4] on gastric acid inhibitory effects of H_2-receptor antagonists, and it is presently presumed there are H_2-receptors in parietal cells.

Study was made on the effects of histamine receptor antagonists on the vascular action of histamine. It has been reported that whereas histamine causes arteriole dilating action and acceleration of capillary permeability, the inhibitory effects of H_1-receptor antagonist are great on the vessels which are distributed in the skeletal muscle and skin[5,16]. Thus, it is said thc H_1-receptor acts more dominantly, but in the intestinally distributed vessels, the inhibiting effects of H_2-receptor antagonist is marked[14,15]. Thus, it is said the H_2-receptor acts more dominantly. It is presumed that the histamine action on the vessels in the stomach presents itself mainly via the H_2-receptor. When our results are viewed with these points in mind, it is felt that the cold restraint ulcer inhibiting effect of diphenhydramine is caused by inhibition of intramucosal histamine release rather than blocking of the intramucosal histamine action at site of the H_1-receptor. It is presumed that the mechanism involved is the powerful cerebral sadative action of the aminoalkylether structure of diphenhydramine. The cold restraint ulcer inhibitory effect of cimetidine is due to blocking at the H_2-receptor site of the action of intramucosal histamine which is released under cold restraint. Prevention of microcirculatory disturbance is considered more important as the mechanism involved than the inhibitory effects of gastric acid secretion.

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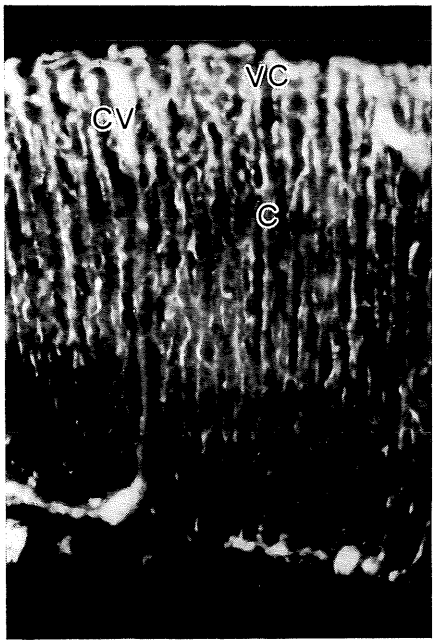

Fig. 4a. Microangiograph in the gastric mucosa of normal rat. The capillary systems are clearly visualized. ×100 Capillaries (C), Venous capillaries (VC), Collecting venule (CV)

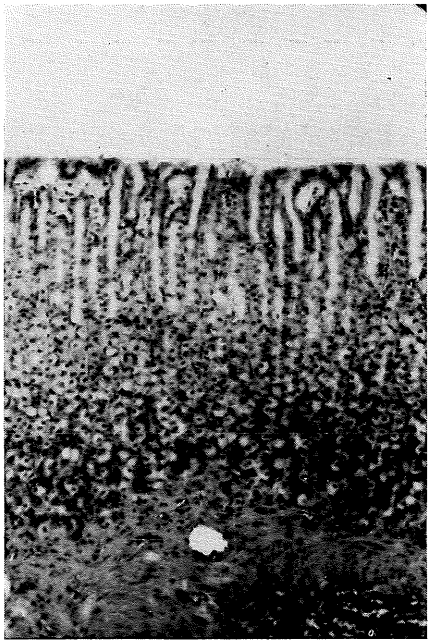

Fig. 4b. Section of normal rat. A few RBC can be seen only in the venous capillaries. H-E. ×100

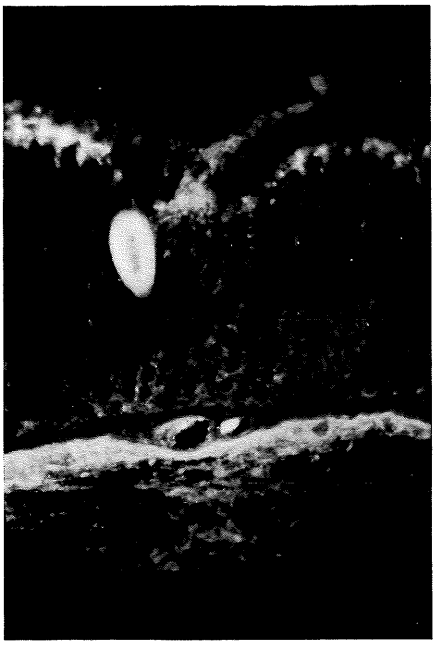

Fig. 5a. Microangiograph of the controls after cold restraint for 60 minutes. Only a few venous capillaries can be vaguely visualized. The bleeding into the stomach are observed. ×100

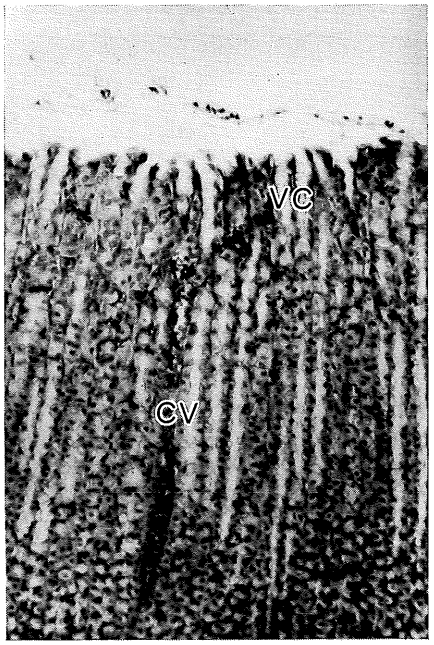

Fig. 5b. Section of the controls after cold restraint for 60 minutes. Neumerous RBC can be seen in the venous capillaries and collecting venules. Venous capillaries (VC), Collecting venures (CV) H-E. ×100

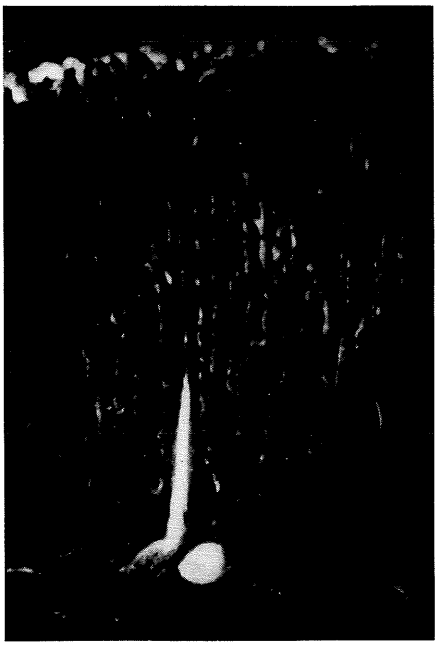

Fig. 6a. Microangiograph of the diphenhydramine 10 mg/kg administered group after cold restraint for 60 minutes. The capillary system are clearly visualized. ×100

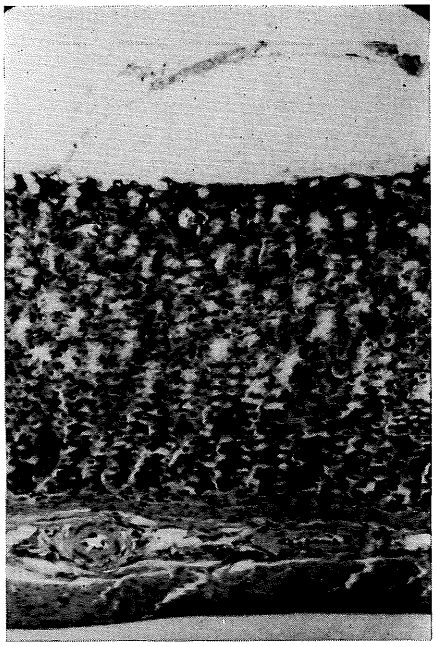

Fig. 6b. Section of the diphenhydramine 10 mg/kg administered group after cold restraint for 60 minutes. The RBC stasis can not be seen. H-E. ×100

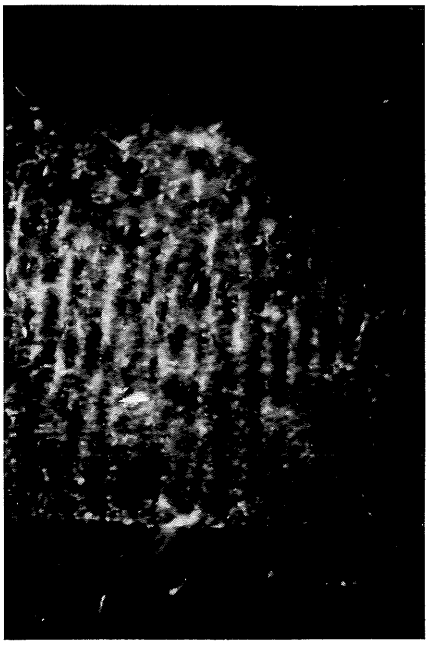

Fig. 7a. Microangiograph of the diphenhydramine 10 mg/kg administered group after cold restraint for 120 minutes. The capillary system are vaguely visualized. ×100

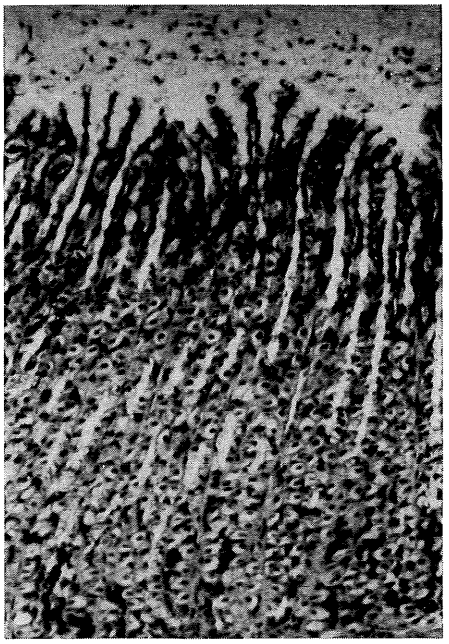

Fig. 7b. Section of the diphenhydramine 10 mg/kg administered group after cold restraint for 120 minutes. The RBC stasis can be seen. H-E. ×100

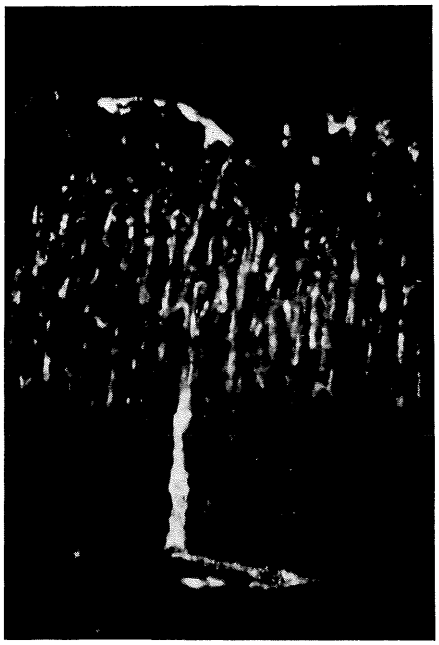

Fig. 8a. Microangiograph of the cimetidine 48 mg/kg administered group after cold restraint for 60 minutes. The capillary system are clearly visualized. ×100

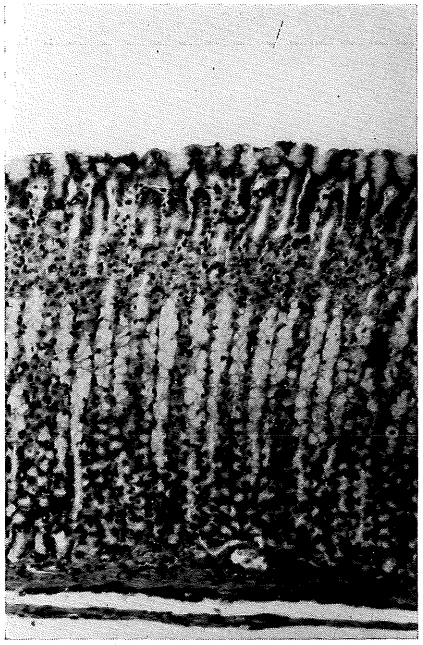

Fig. 8b. Section of the cimetidine 48 mg/kg administered group after cold restraint for 60 minutes. The RBC are stasis can not be seen. H-E. ×100