

The Influence of the Autonomic Nervous System on the Onset of Stress Ulcers—Mainly on the Changes of Biogenic Amine Contents in Brain and Stomach in Cold Restrained Rats—^{*)}

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ABSTRACT

Acute gastroduodenal ulcers are frequently induced by the stress of various type. With a view to examining the influence of the autonomic nervous system on the mechanism of onset of stress ulcers, splanchnicotomy or vagotomy was performed in rats and loaded cold restraint stress for 120 minutes. Subsequently, the contents of biogenic amines in brain and stomach were determined. Further observations on the pH value of the gastric juice and the microvasculature of the gastric mucosa were made, and the following results were obtained.

1. The incidence of gastric ulcers in the control and splanchnicotomized group markedly increased in 60 minutes after stress, whereas in the vagotomized group, it did not show an increase even 120 minutes.
2. As for amines in brain, NA content significantly decreased in 60 minutes, while 5-HT content increased significantly. These changes were fixed, regardless of the cutting of the autonomic nerve or not.
3. Regarding amines in stomach, HA and 5-HT content significantly decreased in the control and splanchnicotomized group.
4. The pH value in gastric juice did not decrease significantly with time up to 120 minutes, in all groups.
5. Impairment on microcirculation in gastric mucosa were suggested in the control and splanchnicotomized group after stress.

Judging from these findings, it was assumed that, for the onset of stress ulcers, NA and 5-HT in brain were associated, whereas in stomach, HA and 5-HT isolated by the vagotonia, rather than the NA isolated by the sympathotonia, were associated. It was further presumed that stress ulcers are caused by the impairments on the microcirculation on gastric mucosa due to the isolation of HA and 5-HT, rather than the increase in the acidity of gastric juice.

INTRODUCTION

In recent years, rapid progress of medicine has

been allowing positive operations on aged persons or patients with severe diseases, and the number of life-saving cases has been in-

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creasing. On the other hand, however, indications for operations or surgical interventions have been expanding, so that surgeons are encountering the onset of acute gastroduodenal ulcers as complications in increasing numbers. Almost all the cases of postoperative acute gastroduodenal ulcers occurred within a week after operation, triggered by the stress of operation or infection, and such cases are regarded as stress ulcers in a wider sense of the term.

Examinations on the reports of the mechanism of the onset of acute gastroduodenal ulcers, or stress ulcers, revealed the following that in 1932 Cushing⁶⁾ summarized his clinical results, pointing out the relation between the occurrence of acute ulcers and vagal nervous system, in 1952 French⁹⁾ suggested from their clinical results that it is important for the onset of stress ulcers that not only the vagal nervous system, but also the sympathetic nervous system, should be associated, and that in 1936 Selye²⁹⁾ pointed out that the hypophyseal-adrenal system is associated for the onset stress ulcers. Thereafter, so many reports have been made on the incidence and onset of stress ulcers. So far, the existence of the hypothalamo-cholinergic system, hypothalamo-sympathetic system, and the hypothalamo-hypophyseal-adrenal system has been known as the routes of passage of stress. The stress loaded on a living organism is passed on to the stomach via these routes, causing stress to occur, to our general belief. It is also considered recently that biogenic amines, which work as vasoactive substances in the capillary, might be working in the central region as neurotransmitter substances. Consequently, these biogenic amines in brain and stomach have been regarded as being important for the onset of stress ulcers^{8,10)}.

The authors therefore performed splanchnicotomy or vagotomy on Wistar-strain rats, and loaded them with cold-restraint as stress, in order to examine the influence of the onset of stress ulcers via the autonomic nervous system among the routes of passage of stress. Subsequently, the following experiments, using such biogenic amines as catecholamine (CA), histamine (HA) and serotonin (5-HT) in brain and stomach, and some findings were obtained. The reports are presented in this paper.

METHODS

1) Experimental animals and experimental groups

Wistar-strain male rats weighing 200–250 g were used for the experiment. The experimental groups consisted of the control group on which no operation was given, the splanchnicotomized group on which the bilateral splanchnicotomy was performed (hereafter to be abbreviated as the Sp group), the vagotomized group on which the bilateral vagotomy was given (hereafter to be abbreviated as the Tv group) and the splanchnicotomized plus vagotomized group on which both splanchnicotomy and vagotomy were performed (hereafter to be abbreviated as Sp+Tv group).

2) Methods for splanchnicotomy and vagotomy

In splanchnicotomy rats were laparotomized by horizontal incision under nembutal anesthesia, and bilateral splanchnic nerve were cut before the celiac ganglion, and the nerve extending to the adrenal was also cut. In vagotomy, the animal was laparotomized with median incision, and bilateral vagal nerve trunks were cut under the diaphragm, and the pyloroplasty was made as well. For splanchnicotomy and vagotomy, the animal was laparotomized with horizontal incision, and then the abovementioned splanchnicotomy, vagotomy and the pyloroplasty, were performed simultaneously. After the operations, animals in all treated groups were bred similarly with those in the control group, and were used for the experiment in 3 weeks after operation.

3) Method for stress

Similarly with Senay's method³⁰⁾, rats were fasted for 24 hours, and then were fixed in metal restraint cages, and housed in the cold-room at 4°C, and were loaded with cold restraint stress for 30, 60 and 120 minutes.

4) Method for the determination of the pH value in gastric juice

Before and after cold restraint was given, the animals were decapitated their blood was let out, the stomachs were extracted, and the monopolar pH electrode (manufactured by Toa Denpa, Model TSC10-A) was inserted from the upper end of the extracted stomachs to determine the pH value in gastric juice.

5) Method for the observation on the occur-

rence of ulcers

After the determination of the pH value in gastric juice, the stomachs were incised on the side of the greater curvature to macrographically observe the lesions of gastric mucosa.

6) Method for the collection of the samples

After the animals were decapitated, the cranial bones were incised and the whole brains were extracted, and then the brains were halved along the longitudinal fissure. Then the thalamus and hypothalamus of a hemisphere were extracted for the determination of CA, and the other hemisphere was extracted for the determination of HA and 5-HT. For the determination of CA, the whole layers of anterior walls of the corpus and antrum, for the determination of HA, the mucosa of the posterior walls of the corpus, and for the determination of 5-HT, the whole layer of the posterior walls of the antrum, were extracted. The samples were put into the deep freezer immediately after collection, and used for quantitative analysis within a week.

7) Method for the determination of CA, HA and 5-HT contents

i) Determination of CA contents

The collected samples were determined in wet weight, homogenized with trichloroacetic acid, and were adsorbed to alumina, and then they were determined with high-performance liquid chromatography¹⁶⁾.

ii) Determination of HA and 5-HT contents

The collected samples were determined in wet weight, deproteinized with 0.4 N perchloric acid, and HA and 5-HT solutions were subjected through Amberlite CG-50 resin for extraction. Then HA was subjected to fluorescence³⁴⁾ with the OPT method under an alkaline condition, and 5-HT, with the OPT method under an acidic condition.

8) Method for the observation of microvasculature in gastric mucosa

Before and after cold restraint, 1 ml 10% FITC-dextran physiological saline solution, with the mean molecular weight of 40,000, was slowly injected intravenously into the femoral vein of the animals, and at the point of time when FITC-dextran was fully circulating in the whole body, the stomachs were immediately extracted, fixed in 20% formalin, and paraffin blocks were manufactured with the dehydrated embedding procedure. The samples thus pre-

pared were sliced thinly to 30 μ , and were observed with fluorescent microscopy (Olympus Model FLM)²³⁾.

RESULTS

1) Changes in the incidence of ulcers

Lesions in stomach caused by cold restraint stress were limited to the corpus in all groups, and there were hemorrhage alone or erosion of Ul-1 in grade. This shall be termed hereafter as acute ulcer. The incidence of ulcers in control group was 33% in 30 minutes after stress, 75% in 60 minutes and 92% in 120 minutes, the incidence being higher in 60 or more minutes, while the incidence in the Sp group was 38% in 30 minutes after stress, 75% in 60 minutes and 88% in 120 minutes, indicating almost the same degree with the control group. The incidence of ulcers in the Tv group and Sp+Tv group was, however, 0%, 13% and 13%, and 0%, 0% and 13%, respectively, in 30 minutes, 60 minutes and 120 minutes after stress, showing marked decreases in the incidence of ulcers at any time, as compared with that in the control and Sp groups (Table 1).

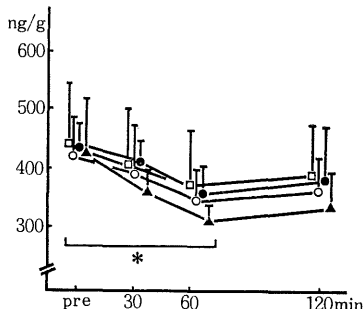
Table 1. Incidence of gastric hemorrhage and erosion on cold restrained rats

restraint time group	30 min	60 min	120 min
Cont.	33% (4/12)	75% (9/12)	92% (11/12)
Sp.	38 (3/ 8)	75 (6/ 8)	88 (7/ 8)
Tv.	0 (0/8)	13 (1/ 8)	13 (1/ 8)
Sp.+Tv.	0 (0/8)	0 (0/ 8)	13 (1/ 8)

2) Changes in amine contents in brain

i) Changes in NA content

NA content before stress was 442.2 ± 26.4 ng/g ($M \pm SD$) in the control group, 423.6 ± 68.4 ng/g in the Sp group, 421.7 ± 55.8 ng/g in the Tv group and 452.8 ± 68.7 ng/g in the Sp+Tv group. In 60 minutes after stress when the incidence of ulcers was elevated, the figures were significantly decreased to 346.8 ± 28.0 ng/g, 317.5 ± 34.6 ng/g, 353.4 ± 52.2 ng/g, and 372.3 ± 52.2 ng/g, respectively, as compared with those before stress, continuing the decrease thereafter (Fig. 1).



●: Cont. (n=10) ▲: Sp. (n=6) ○: Tv. (n=6) □: Sp.+Tv. (n=6)

Fig. 1. Chronological changes of noradrenalin content in brain after cold restraint

* Significance of the difference between means of the pre-test and restraint value within each study group ($M \pm SD$, $p < 0.05$)

ii) Changes in DA content

DA content before stress was 670.4 ± 12.4 ng/g in the control group, 661.4 ± 134.1 ng/g in the Sp group, 652.1 ± 153.2 ng/g in the Tv group, and 653.7 ± 122.9 ng/g in the Sp+Tv group, showing a mild decrease in 30 minutes after stress in all the group and increasing slightly thereafter. These changes were, however, not significant, as compared with those before stress at any time all groups (Fig. 2).

iii) Changes in 5-HT content

5-HT content in each group before stress was 230.6 ± 41.5 ng/g in the control group, 217.0 ± 38.7 ng/g in the Sp group, 181.6 ± 62.7 ng/g in the Tv group and 204.4 ± 61.5 ng/g in the Sp+Tv group. These figures were significantly increased in 60 minutes after stress to 331.8 ± 40.0 ng/g in the control group, 314.6 ± 68.6 ng/g in the Sp group, 300.0 ± 86.5 ng/g in the Tv group and 306.5 ± 58.8 ng/g in the Sp+Tv group, respectively ($p < 0.05$), increasing gradually thereafter (Fig. 3).

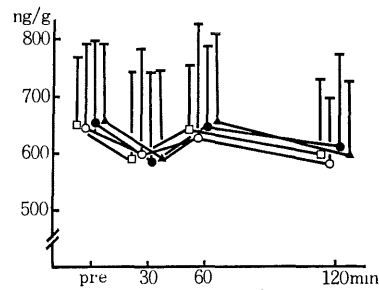
iv) Changes in HA content

HA content before stress was 66.4 ± 17.4 ng/g in the control group, 62.6 ± 6.0 ng/g in the Sp group, 68.4 ± 10.1 ng/g in the Tv group, and 69.3 ± 12.6 ng/g in the Sp+Tv group. After stress, however, the figures were increased slightly in 30 minutes in all groups, gradually decreasing until 120 minutes afterwards, but these changes were not significant, as compared with those before stress (Fig. 4).

3) Changes in amine contents in stomach

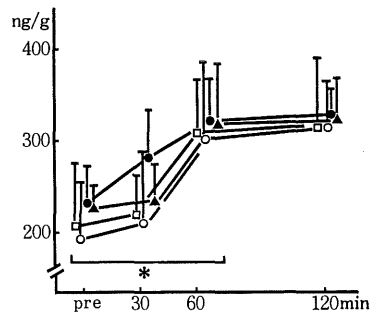
i) Changes in NA content

NA content before stress were 210.5 ± 22.6



●: Cont. (n=10) ▲: Sp. (n=6) ○: Tv. (n=6) □: Sp.+Tv. (n=6)

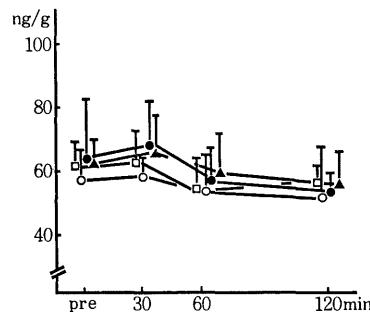
Fig. 2. Chronological changes of dopamine content in brain after cold restraint ($M \pm SD$)



●: Cont. (n=10) ▲: Sp. (n=6) ○: Tv. (n=6) □: Sp.+Tv. (n=6)

Fig. 3. Chronological changes of serotonin content in brain after cold restraint

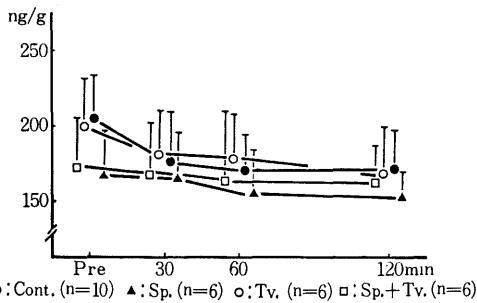
* Significance of the difference between means of the pre-test and restraint value within each study group ($M \pm SD$, $p < 0.05$)



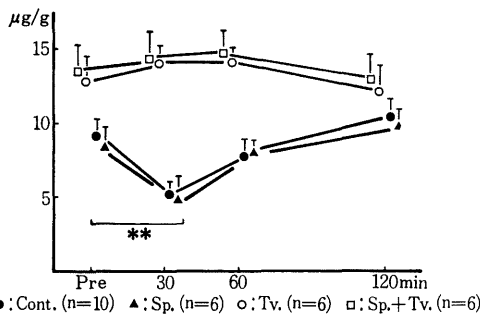
●: Cont. (n=10) ▲: Sp. (n=6) ○: Tv. (n=6) □: Sp.+Tv. (n=6)

Fig. 4. Chronological changes of histamine content in brain after cold restraint ($M \pm SD$)

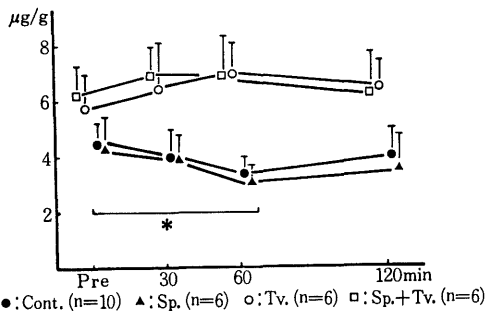
ng/g in the control group, 167.9 ± 30.1 ng/g in the Sp group, 200.3 ± 29.3 ng/g in the Tv group and 174.0 ± 34.8 ng/g in the Sp+Tv group, and the decrease was noted in the Sp and Sp+Tv groups, as compared with the control. These figures after stress showed a decreasing tendency in 30 minutes in the control and Tv groups, indicating no changes thereafter. In the Sp and Sp+Tv groups,



●: Cont. (n=10) ▲: Sp. (n=6) ○: Tv. (n=6) □: Sp.+Tv. (n=6)
Fig. 5. Chronological changes of norepinephrine content in stomach after cold restraint (M±SD)



●: Cont. (n=10) ▲: Sp. (n=6) ○: Tv. (n=6) □: Sp.+Tv. (n=6)
Fig. 6. Chronological changes of histamine content in stomach after cold restraint
 ** Significance of the difference between means of the pre-test and restraint value within each study group (M±SD, p<0.01)



●: Cont. (n=10) ▲: Sp. (n=6) ○: Tv. (n=6) □: Sp.+Tv. (n=6)
Fig. 7. Chronological changes of serotonin content in stomach after cold restraint
 * Significance of the difference between means of the pre-test and restraint value within each group (M±SD, p<0.05)

however, almost no changes were noted after stress (Fig. 5).

ii) Changes in HA content

HA before stress was 8.9±1.2 μg/g in the control group, 8.6±1.1 μg/g in the Sp group, 12.8±1.8 μg/g in the Tv group and 13.2±2.1 μg/g in the Sp+Tv group. These contents

after stress significantly decreased in the control and Sp groups to 5.2±0.8 μg/g and 5.0±1.6 μg/g, respectively (p<0.01), gradually increasing thereafter until 120 minutes afterwards. In the Tv and Sp+Tv groups, however, almost no changes were observed until 60 minutes after stress, showing a slight decrease in 120 minutes (Fig. 6).

iii) Changes in 5-HT content

5-HT content in stomach before stress was 4.5±0.8 μg/g in the control group, 4.3±1.1 μg/g in the Sp group, 5.9±1.1 μg/g in the Tv group and 6.1±2.1 μg/g in the Sp+Tv group, while 5-HT content after stress in the control and Sp groups was 3.4±0.6 μg/g and 3.0±0.6 μg/g, respectively in 60 minutes after stress when the incidence of ulcers is increased, indicating a significant decrease (p<0.05), as compared before stress. In the Tv and Sp+Tv groups, on the other hand, the figures were slightly increased in 60 minutes after stress, showing almost no changes thereafter (Fig. 7).

4) Changes in the pH values in the gastric juice

The pH values in gastric juice before stress were 2.3±0.6 in the control group, 2.4±0.9 in the Sp group, 3.6±0.8 in the Tv group and 3.5±0.9 in the Sp+Tv group. It was noted that the pH values in gastric juice was significantly elevated (p<0.01), as compared with the control, after vagotomy. The pH values in gastric juice in 120 minutes after stress in the control and Sp groups were 1.9±0.3 and 1.7±0.6, respectively, while in the Tv and Sp+Tv groups, the values remained elevating to 3.0±0.6 and 3.2±0.4, respectively in 120 minutes (Table 2).

Table 2. Chronological changes of pH in gastric juice after cold restraint

restraint time group	pre-test	30 min	60 min	120 min
Cont. (n=10)	2.3±0.6	2.2±0.3	2.0±0.4	1.9±0.3
Sp. (n=6)	2.4±0.9	2.1±0.6	1.8±0.4	1.7±0.6
Tv. (n=6)	3.6±0.8	3.4±0.7	3.3±0.8	3.0±0.6
Sp.+Tv. (n=6)	3.5±0.9	3.5±1.0	3.4±0.8	3.2±0.4



Fig. 8. Microangiograph in the gastric mucosa of normal rat. The capillary systems are clearly visualized. $\times 100$ Capillaries (C), Venous capillaries (VC), Collecting venule (CV)



Fig. 10. Microangiograph of the splanchnicotomized rat after cold restraint for 60 minutes. Only a few venous capillaries can be visualized. $\times 100$

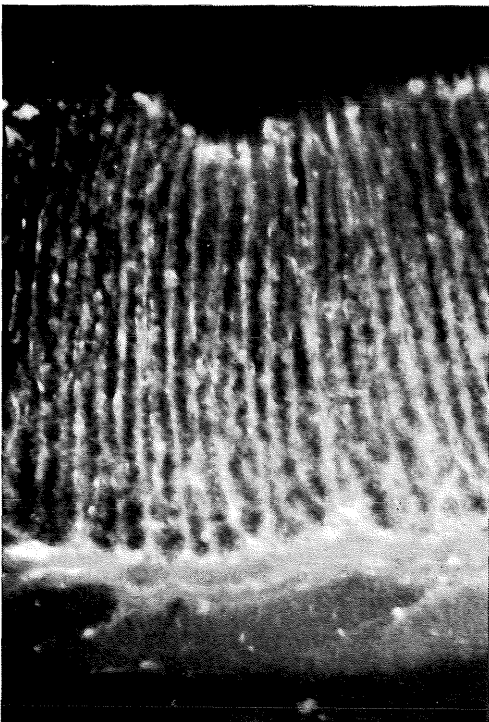


Fig. 9. Microangiograph of the control rat after cold restraint for 60 minutes. Only a few venous capillaries can be visualised. $\times 100$

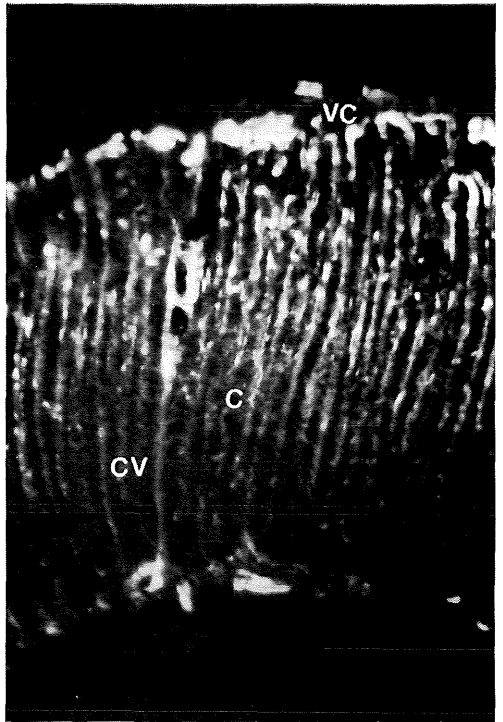


Fig. 11. Microangiograph of the vagotomized rat after cold restraint for 60 minutes. The capillary systems are clearly visualized. $\times 100$ Capillaries (C), Venous capillaries (VC), Collecting venule (CV)

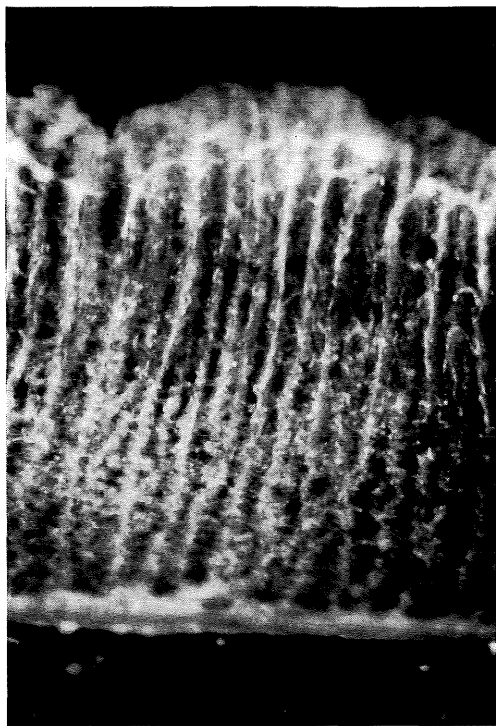


Fig. 12. Microangiograph of the splanchnicotomized and vagotomized rat after cold restraint for 60 minutes. The capillary systems are clearly visualized. $\times 100$

5) Findings in microvasculature in gastric mucosa

Since the onset of ulcers was significantly increased in the control and Sp groups in 60 minutes after stress, examinations on microvasculature in all groups were made in 60 minutes after stress. It was observed in the control group before stress that capillaries ascending meandering through the mucosal layers to the surface layer, capillaries transversing the surface layers of the mucosa, and collecting venules descending almost perpendicularly through the mucosa, were existing (Fig. 8). In 60 minutes after stress in the control group, however, capillaries in surface layers of the mucosa were barely noted, but other capillaries and collecting venules in the mucosa were not observed (Fig. 9). On the other hand, in 60 minutes after stress in the Sp group, capillaries were barely noticed similarly with those in the control in 60 minutes after stress (Fig. 10). But in the Tv and Sp+Tv groups, capillaries ascending meandering in the mucosa, capillaries in surface layers, and collecting venules, were clearly

noted, similarly with those in the control before stress (Fig. 11, 12).

DISCUSSION

Stress ulcers have long been well-known as Curling's ulcer after burn, and Cushing's ulcer after cerebral disease. It has been reported in recent years that stress ulcers occur in high frequency during the courses of various diseases or after operations^{11,12}. There have been voluminous reports on the mechanism of onset of stress ulcers, examining the etiology in terms of various factors, existing in the locality of the gastric mucosa, that is, the matrix of the onset of ulcers, such as aggressive factors like gastric acid and pepsin, or such as defensive factors like mucus, tissue resistance and microcirculation in the gastric mucosa^{5,24,31}. French and Porter⁹ and Matsuo et al.²² pointed out, however, that the influence of the central nervous system acting on the focus in the gastric mucosa is important for the onset of stress ulcers. More in particular in recent years, biogenic amines in brain, which are being clarified clearly as the neurotransmitter substances, have been regarded as central factors for the onset of stress ulcers^{16,26,28}.

The authors therefore studies the relation between biogenic amines in brain and the onset of stress ulcers. It has been reported that the changes in NA content in brain under stress are decreased, according to some researchers^{3,26}, or increased or unchanged, according to others^{17,32}. Our results indicated that NA content in brain was markedly decreased in 60 minutes under stress, which showed that it was isolated under stress. It can easily presumed that this isolation of NA in the thalamus and hypothalamus, which are the center of the autonomic nervous system, brought about the tonus of the sympathetic nervous system, acting on the peripheries. Ban² pointed out, however, that in rabbits sympathetic nervous fibers govern vagal nervous fibers in the central nervous system and inhibit the actions of the latter. On the other hand, Fujiwara et al.¹⁰ reported that the decrease in NA content in brain after stress relatively caused the vagotonia and that the further exogeneous increase in NA content in brain clearly inhibited the occurrence of stress ulcers. Although acetylcholine content in brain, which has been considered to reflect

the vagotonia, has not been determined, the authors too have been presuming that the decrease in NA content in brain after stress may weaken the actions of the tonus of sympathetic nerves, which inhibits the tonus of vagal nerves, thus bringing about the vagotonia. Accordingly, it was considered that the changes in NA content in brain might be closely associated with the onset of stress ulcers through the autonomic nervous system.

The subsequent study was made on the relation between the changes in 5-HT content in brain at stress and the onset of ulcers. The changes in 5-HT content in brain under stress have been reported, similarly with those of NA content, contradictorily according to the differences of the methods for stress^{3,8)}. According to the results obtained by the authors, 5-HT content in brain was markedly increased in 60 minutes after stress, unlike NA content in brain. The actions of intracerebral serotonergic system include the motor inhibitory action and thermogenesis inhibitory action, but it has been considered to act competitively with those of intracerebral adrenergic system. Accordingly, the increase in 5-HT content in brain may have worked competitively with the decrease in NA content. However, there have been some reports^{3,35)} stating that 5-hydroxyindol acetic acid (5-HIAA), a metabolite of 5-HT, was increased in brain under stress, so that the increase in 5-HT content observed by the authors might be due to the acceleration of the velocity of metabolism, rather than due to the elevation of the synthesis and storage of 5-HT content.

Incidentally, it has been pointed out in some reports^{18,35)} that 5-HT content in brain is, together with NA content, associated with the release of corticotropin-releasing hormone (CRH), which isolates adrenocortico tropic hormone (ACTH). In consideration of this finding, it can be considered that 5-HT content in brain is acting to maintain homeostasis, which is the general adaptation syndrome as pointed out by Selye, under stress. In the present experiment, therefore, the mechanism of the association of 5-HT content in brain in the onset of ulcers could not be clarified, but it might be suggested that it is related, together with NA content in brain, with the onset of stress ulcers.

The central actions of DA was found to be spontaneous movements through the extrapyramidal system, which is still in a lower position than the central system in which NA or 5-HT is associated, as well as the secretion inhibitive actions for mammatropic hormone and luteinizing hormone among hormonal actions in the pituitary anterior lobes. It has been considered that the central actions of HA include the thermogenesis action, together with 5-HT, but other actions remain unclarified. Therefore, the changes in DA content and HA content in brain having these physiological actions, under stress load, were not significant, similarly with the reports by some researchers^{3,26)}. It is estimated therefore that, judging from the central actions of DA or NA, and their changes after stress, the possibility of the direct association of DA or HA content in the onset of stress ulcers, might be slim.

The next subject for study was the relation between the changes in biogenic amines in brain under stress and the autonomic nervous system. As has been stated previously, sympathetic nervous fibers were recognized to be extending not only to the nucleus of sympathetic nerves in peripheries, but also in the nucleus of vagal nerves, and the cell bodies of 5-HT existing in the brain stem extend their termini in the nucleus of sympathetic nerves in the spine partially, but the reports on the relation between the changes in biogenic amines in brain and the autonomic nervous system have been very few. Nagase²⁶⁾ reported that amines in brain were decreased by adrenalectomy, but that no influence by vagotomy was received, whereas Ikeda¹⁵⁾ stated that vagotomy caused the response of the central system to stress to change, and that the changes in NA content in brain under stress were different. The results obtained by the authors revealed that the changes in any of amines in brain were regular, irrespective of the cutting of the autonomic nerve or not. Generally speaking, the number of the centripetal fibers is by far smaller than the number of the centrifugal fibers, and their are said to be nothing but the conduction of visceric algesis. Accordingly, the cutting of the autonomic nervous system is considered from its anatomical structure and physiological action may hardly give influence on the conduction of stress to the brain or on the response

of the central system to stress.

It is presumed from these findings that, in case stress is loaded on a living organism, the response of the central system to stress and the conduction of the stress to peripheries is regular, regardless of the cutting of the autonomic nerve or not, and that among biogenic amines in brain, NA and 5-HT content are important as central factors working on the onset of stress ulcers.

The next discussion is the gastric mucosal factors influencing on the onset of stress ulcers. Let us examine the influence of biogenic amines in stomach on the occurrence of stress ulcers. It is well-known that the administration of active amines existing abundantly in the gastric walls of rats, such as CA, HA or 5-HT, produces gastric ulcers. Hori¹⁴⁾ reported that the administration of NA and 5-HT concomitantly, not either of them alone, produced gastroduodenal ulcers. It has also been recognized fluorescent-histochemically and quantitatively that biogenic amines in stomach are altered under stress^{10,38)}. Therefore, an important influence of biogenic amines in stomach on the onset of stress ulcers has been suggested, but it is not clear which of these amines is associated with the onset of stress ulcers. According to our results, NA content in stomach was isolated by the tonus of the sympathetic nerve, and NA synthesis was inhibited by the cutting of sympathetic nerve. Furthermore, the tonus of the sympathetic nerve was presumed to have occurred, judging from the changes in NA content in stomach, within 30 minutes after stress. However, no definite relation between NA isolation and the incidence of ulcers was found. HA and 5-HT contents in stomach were significantly decreased in 30 to 60 minutes after stress, and at that period remarkable occurrence of ulcers were noted. When vagotomy was performed, however, the decrease in both amines was inhibited, and a marked decrease in the incidence of ulcers was found. This finding was similar with the reports of Kahlson¹⁹⁾ and Ahlman et al.¹⁾ that HA and 5-HT contents in stomach were isolated by the stimulation of the vagal nerve. As has been explained so far, the reports that the administration of HA and 5-HT induced occurrences of gastric ulcers are innumerable. It is presumed accordingly that HA and 5-HT which possess

ulcer-producing actions under stress are isolated by the tonus of the vagal nerve, causing stress ulcers to occur.

As for the time of appearance of the tonus of the autonomic nerve under stress, Leonard²¹⁾ pointed out after his experiment of central nervous system stimulation using cats and dogs that the tonus of the sympathetic nerve occurred first at an early stage of stress, followed by the tonus of the parasympathetic nerve. Klein²⁰⁾ followed it up in more detail, using rats in restraint stress, reporting that the tonus of the sympathetic nerve was produced first, and in 3 hours the tonus of the parasympathetic nerve followed. Although the outcome obtained by the authors was only after the examination of the changes in biogenic amines in stomach, the tonus of the parasympathetic nerve occurred in 30 minutes after stress, and the tonus of the sympathetic nerve must have occurred previously. The difference of time of appearance of the tonus of the autonomic nerve is presumed to have occurred by our method being double stress of cold and restraint.

Examination of relation between biogenic amines in stomach and the onset of stress ulcers from these findings pointed to a close association of HA and 5-HT isolated from the vagotonia, rather than caused by NA isolated from the sympathicotonia occurring immediately after stress.

The influence of the secretion of gastric acid on the onset of stress ulcers was also studied. In 1910 Schwarz mentioned "No acid, no ulcer", and since then many reports have been made, maintaining that the secretion of gastric juice is accelerated when stress ulcers are occurring, and attributing the acidity of gastric juice to the onset of acute ulcers as an aggressive factor. For example, Klein et al.²⁰⁾ reported that, when restraint stress was loaded on rats, the acidity of their gastric juice was increased, while Brodie et al.⁴⁾ argued contradictorily that the acidity of gastric juice was decreased with the stress model similarly prepared with our model. The results of the authors made agreement with those by Brodie et al., and in the control and Sp groups where the incidence of ulcers was high, the pH value of gastric juice showed but a mild decrease. The results may not deny a possibility of back diffusion pointed out by Davenport et al.⁷⁾ Merserou et al.²⁵⁾ reported,

however, that the infusion of an acid in a high concentration into the stomach did not produce ulcers on the normal mucosa, although ulcers occurred when the gastric mucosa had been impaired or there had been bile reflux. It may be considered from these reports that the increase of the acidity of gastric juice may be a factor aggravating the produced ulcers, but that it may not become a stress-producing factor.

Last but very important is the discussion of microcirculation in the gastric mucosa as the defensive factor for the mucosa. In 1853 Virchow proposed the vascular-impairment theory as the etiology of peptic ulcers, and since that time, association of microcirculation in the gastric mucosa with the occurrence of acute ulcers has been considered. As has been known well, NA, a vasoactive amine, brings about ischemia in the surface layer of the mucosa by the constriction of arterioles, while HA and 5-HT dilate arteriole, but they constrict venules, eventually producing hyperemia in the capillaries of the mucosa, and stasis too. It can be easily presumable that either ischemia or stasis will cause hypoxia on the surface layers of the mucosa, and ulcers occur as a natural consequence. Further, it has been pointed out nowadays that the A-V shunt supposedly existing in gastric submucosa is ordinarily closed, whereas it is opened by the administration of amines or under stress, bringing about impairments on the microcirculation in the gastric mucosa¹⁹⁾. In the results of authors where the FITC-dextran method²⁰⁾ was adopted for the observation of the microcirculation in the gastric mucosa, such impairments were suggested in 60 minutes after stress when the incidence of ulcers was markedly increased. These impairments were also induced after the splanchnic nerve was cut and stress was loaded, whereas vagotomy and stress load did not produce the impairments. Judging from the changes in biogenic amines in stomach as mentioned before, the impairments on microcirculation under stress are supposed to be due to the HA and 5-HT isolated by the vagotonia.

Ogawa²¹⁾ examined the impairments on microcirculation in cold restrained rats in detail, using the H-E staining method, and pointed out that the impairments on microcirculation under stress was mainly due to the stasis in capillaries in the mucosa caused by HA. It is also said

that HA and 5-HT are acting on the closing and opening of the A-V shunt, but this observation with our method is extremely difficult. Consequently, our results failed to clarify whether the impairments on microcirculation under stress are directly due to the actions of vasoactive amines or due to the actions via shunt. Further studies are required to clarify whether this was due to the synergism of HA and 5-HT or not. However, it could be presumed that both these amines cause the impairments on microcirculation in the gastric mucosa, and are closely associated with the etiology of stress ulcers.

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