The pathogenesis of gastric ulceration induced by drugs or experimental stress is not clear. Although many reports have been published about the influence of biogenic amines on the production of experimental ulcer in animals, few of them are available.

It is well known that gastric histamine (Hm) and 5-hydroxytryptamine (5-HT) are found abundantly in the fundal and the antral mucosa of rats. In the present study, we investigated the relationship between the occurrence and severity of gastric lesions and the population of fluorescent enterochromaffin cells (EC cells), which contain 5-HT, in the gastric mucosa of rats treated with reserpine or cold restraint stress. There may be a difference in mechanism between reserpine-induced and stress-gastric lesions, both of which are observed in the fundal (glandular) mucosa of rats.

The mucosal content of 5-HT was estimated quantitatively for the reliability of quantitation of EC cells. Furthermore, the mucosal content of gastric Hm was also quantitated, although it is still doubtful whether Hm is a final mediator for gastric acid secretion.
MATERIALS AND METHODS

Using 48-hour fasted male Wister rats, weighing 180 g, reserpine (10 mg per kg)-induced gastric lesions and cold-restraint stress induced gastric lesions were produced. The rats were divided into groups on the basis of the following intervals, 0.5, 1.0, 1.5, 3, 6, and 9 hr after the treatment. The experiment was then performed on each group. The rats treated with reserpine or stress were sacrificed by decapitation, and their stomachs then resected and incised along the greater curvature. Each opened gastric mucosa was carefully checked for the presence of gastric lesions. The glandular mucosa was utilized for the evaluation of Hm, while the antral mucosa was utilized for the quantitation of 5-HT and the identification of EC cells. Falleck and Hillarp's method was used for the identification of EC cells, while Bogdanski's and Shore's method were employed for the evaluation of 5-HT and Hm, respectively.

The number of EC cells in a square unit (1 mm² × 4 micron) was calculated by means of an ocular-eye grid under Nikon fluorescent microscopy (×100).

Preliminary Study

The antral mucosa was divided into 9 areas for study of the distribution of EC cells. The number of EC cells was calculated in each area, the results showing that a nearly equal number of EC cells was rather diffusely distributed. Therefore, the mucosal specimens of the antral mucosa of the lesser curvature were utilized for this study.

The gastric lesions that had formed were classified into the following 3 grades; U I, U II and U III, according to the principle of severity of gastric lesion as being evaluative of the ulceration index.

Since very little difference between the number of EC cells in 12, 24 and 48 hour-starvation rats was observed, it was decided to employ 48-hour rats for this study.

In order to understand the correlation between the number of EC cells and

Relationship Between the Content of Mucosal 5HT & the Number of EC Cells

![Relationship Between the Content of Mucosal 5HT & the Number of EC Cells](image)

Fig. 1. A significant correlation between the mucosal content of 5-HT and the number of EC cells can be observed.
the mucosal content of 5-HT, the mucosal content of 5-HT was quantitated, as mentioned before. The data showed a significant statistical correlation between the number of the cells and the mucosal content of 5-HT (Fig. 1).

The doses of reserpine were administered intraperitoneally in doses of 0.1, 1.0, 5, 10, 20 mg per kg in order to check the dose-dependency of the drug. With increase in the dose of reserpine, a corresponding increase in the incidence and severity of gastric lesions was observed, while there was a decrease in the number of EC cells and mucosal amines. Since the number of EC cells and the mucosal content of Hm was lowest when 10 mg per kg of reserpine was administered, this dose was employed in the experiment. It is long been known that Hm might be one of the local chemostimulators (such as acetylcholine and gastrin) in rats, although it is still questionable whether Hm is a final mediator for gastric acid secretion. As we are in agreement with the hypothesis that Hm is one of the local chemostimulators of the gastric acid secretion, the mucosal content of gastric Hm was estimated quantitatively as a marker of gastric acid secretion.

RESULTS AND DISCUSSION

Gastric lesions were already observed during the initial stage of 30 to 60 min after administration of reserpine, and, further, the percentage of incidence and severity of the lesions increased as time passed. Although a decrease in the number of EC cells was observed in the first thirty to ninety minutes after administration of reserpine, no constancy in the decrease was observed (Fig. 2). The mucosal content of gastric Hm decreased during the initial thirty to sixty minutes

![Graph](image1.png)

![Graph](image2.png)

**Fig. 2.** Gastric lesions were observed from the first thirty minutes to nine hours after treatment with reserpine or cold-restraint stress. Although decrease in the number of EC cells was observed in both experimental ulcer, in the case of reserpine poor constancy in the decrease was noticed during the first thirty to ninety minutes after treatment.
after administration of reserpine in association with the increased percentage of gastric lesions (Fig. 3). This suggests that during the initial stage of the experiment, mucosal Hm might be more involved in the production of gastric lesions caused by reserpine than 5-HT.

No remarkable change could be observed in the percentage of incidence and severity of gastric lesions at 3 to 9 hr after libration of stress. Although the number of EC cells decreased significantly (p < 0.05) during the initial thirty to ninety minutes of the stress (Fig. 2), severe lesions (U II to III) comparable to those observed in the case of reserpine did not occur. These observations suggest that reserpine might be more ulcerogenetic than stress. Any significant change in the mucosal content of Hm during the initial thirty to ninety minutes after libration of stress, would suggest that gastric Hm might not influence the production of gastric lesions that much during the initial stage of the stress (Fig. 3).

It has long been remarked that when reserpine is administered to rats a marked lowering of gastric Hm concentration and gastric acid secretion results. This effect of reserpine arises from a centrally vagal stimulation. Therefore, it is meaningful to examine the influence of chemical vagotomy induced by atropine on the production of gastric lesions and to examine the population of EC cells as well as the mucosal content of gastric amines. In the experiment in which atropine was applied to reserpine cases, not only both the decrease in number of EC cells and the release of mucosal Hm-content were blocked but also the production of gastric lesions was inhibited (Fig. 4). In contrast to these observations, when atropine was used in stress cases although decrease in the number of EC cells and the release of mucosal Hm were blocked, the production of gastric lesions was only slightly inhibited. (Fig. 4). These observations suggest that vagal stimul-
BIOGENIC AMINES IN RAT ULCER

The release of mucosal 5-HT (EC cells) and histamine is not only blocked, but the production of gastric lesions caused by reserpine is also inhibited. The left column and ●: without atropine, the right column and ○: with atropine.

Among the many different hypothesis concerning the role of gastric amines in the pathogenesis of experimental ulcer in the rat, the hypothesis that the formation of gastric ulcer caused by reserpine originates from the depletion of catecholamines in the gastric mucosa of rats might be correct. In the present study, we also examined the action of catecholamines, especially gastric dopamine, in the production of gastric lesions caused by reserpine and stress. It has been demonstrated that pharmacological activities can be mainly attributed to catecholamines which are formed centrally and peripherally. When the dose of 500 mg per kg of L-dopa was administered to rats treated with reserpine, no gastric lesions were observed during the initial stage of 30 min. However when in order to examine the difference in pathogenesis between reserpine and stress cases a 500 mg per kg dose of L-dopa was administered to rats treated with cold restraint stress, the production of gastric lesions was not inhibited. These observations indicate that the formation of gastric lesions by reserpine could be antagonized by L-dopa involved in the metabolism of catecholamines. However, the dose of L-dopa required to produce such an effect is much higher than a dose required to produce physiological action. When the dose of 50 mg per kg of L-dopa was administered
to reserpine-treated rats, poor protective action against the formation of gastric lesions was observed (Fig. 5). A clear mechanism for the protective role of catecholamines against gastric lesions caused by reserpine still remains unresolved in this study. The protective action of L-dopa against gastric lesions caused by stress was poor, suggesting that there might be many factors in the pathogenesis of this kind of gastric lesion. From these observations it seems likely that in the rat the mucosal content of the stomach catecholamines (mainly dopamine) plays an important role in the pathogenesis of gastric lesions caused by reserpine. However, this does not hold true in the case of gastric lesions caused by stress. Moreover, restoration of the level of dopamine can directly or indirectly antagonize the formation of gastric lesions.

With regard to norepinephrine, although it was examined whether the drug has some influence on gastric lesions caused by reserpine or stress, very little protective action such as that in the case of dopamine was observed. This is an important problem to be resolved in the future. Thus, there are many problems to be elucidated with regard to the possible role of catecholamines in the pathogenesis of experimental gastric lesions caused by reserpine.

From these observations, it seems likely that 5-HT influences the formation of gastric lesions caused by reserpine or stress. Therefore, the influence of parachlorophenylalanine (pCPA) (which blocks the conversion of 5-hydroxytryptophan (5-HTP) to 5-HT) on the production of gastric lesions was examined in order to determine whether 5-HT plays an actual role in the formation of gastric lesions caused by reserpine or stress. With pCPA administration, the formation of gastric lesions caused by reserpine was inhibited, while formation due to stress was not. Further, there was a decrease in the number of EC cells. This suggests that 5-HT could play an important role in the formation of gastric ulceration by reserpine.

On the other hand, the number of EC cells increased greatly when 5-HTP, a precursor of 5-HT, was administered to rats treated with reserpine. However, the percentage of gastric lesion incidence caused by reserpine was not that much higher than that in non-treated rats (reserpine-treated alone). The observations
suggest the complexity of the involvement of the gastric amines in the formation of gastric lesions.

In conclusion, the exact role of gastric EC cells (5-HT) in the pathogenesis of experimental gastric ulcer caused by reserpine or stress will be made clear when we discover what is the precise physiological role of gastric 5-HT, but not intestinal 5-HT and why so many EC cells are present in the antral mucosa where some endocrine cells containing polypeptides (mainly G cells) are also abundant in man and rats.

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ULTRASTRUCTURE AND CYTOCHEMICAL STUDIES ON HUMAN TUMORS SECRETING CATECHOLAMINES

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Correlative ultrastructural and biochemical studies on 38 human neurogenic tumors, adrenal medulla and sympathetic ganglia revealed the following findings: Firstly, this group of tumors has an ability to synthesize catecholamines (CA). Secondly, the ultrastructure of tumors resembles that of their normal tissue counterparts of various differentiations. Multidirectional differentiation in the tumor is common. Thirdly, the CA granules in pheochromocytoma and paraganglioma were similar to those in adrenal medulla of human and rodent. Large cored, small cored and small clear vesicles in all neurogenic tumors resemble those in sympathetic ganglia and nerve endings. Granular resemblance suggested the similarity of CA storage in tumors and in control tissues. Fourthly, double fixation with the use of aldehyde and osmium revealed that the human adrenal medulla have cells with ellipsoidal granules (E granule) as well as cells with more or less rounded granules (R granule). Fifthly, double fixation revealed that pheochromocytomas have three kinds of cells containing E granules, R granules and granules with dense, peripherally located cores (dp granules) that resemble the NA granules of rat. Sixthly, chemical assay showed the predominance of E and/or dp cells in all the NA dominant tumors. Chemically mixed tumors showed a mixed type of dp/E and R cells. One A dominant tumor was R dominant. This suggests that E and dp granules contain NA and that R granules contain A in pheochromocytomas. The E cells in human adrenal medulla also appear to be of the NA storing type.