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Histamine and Serotonin in the Gastric Erosions of Rats Dead from Exposure to Cold: A Histochemical and Quantitative Study*

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Summary. Rats were kept at —20°C until they died. Stomach erosions were found in every rat exposed to cold. Histamine and serotonin were visualized by fluorescence microscopy and their quantities were measured fluorometrically.

Histamine fluorescence had disappeared in the erosion region from mucosal mast cells and enterochromaffin-like cells. Submucosal mast cells had retained full fluorescence and showed no degranulation. The amine content showed an insignificant decrease. Serotonin fluorescence had also disappeared from the mucosal mast cells in the erosion, but was visible in enterochromaffin cells and submucosal mast cells beneath the erosion. Degranulation of mucosal mast cells was confirmed in sections stained by toluidine blue. Gastric serotonin level was significantly lower in the test group than in the control group (0.58 ± 0.23 μg/g versus 0.99 ± 0.28 μg/g). The results confirmed the effectiveness of cold exposure in causing gastric erosion in the rat. Direct evidence of the release of histamine and serotonin from the mucosal cells during formation of erosions was obtained.

Zusammenfassung. Ratten wurden bei —20°C gehalten bis sie starben. Magenerosionen wurden in jeder der Kälte ausgesetzten Ratte gefunden. Histamin und Serotonin wurden sichtbar gemacht mittels der Fluorescenzmikroskopie, und ihre Mengen wurden fluorometrisch gemessen.

Histamine-Fluorescenz war in der zersetzten Zone der Schleimhautmastzellen und enterochromaffinähnlichen Zellen verschwunden. Untere Mastzellen enthielten volle Fluorescenz und zeigten keine Degranulation. Der Amingehalt zeigte einen unbedeutenden Abfall. Serotonin-Fluorescenz war gleichfalls aus den Schleimhautmastzellen verschwunden, war aber sichtbar in Enterochromaffinzellen unterhalb der Zersetzung. Degranulation der Schleimhautmastzellen wurde bestätigt in Schnitten, die mit Toluidinblau gefärbt waren. Der gastrische Serotoningehalt war bemerkenswert niedriger in der Testgruppe als in der Kontrollgruppe ($0{,}58 \pm 0{,}23$ μg/g versus $0{,}99 \pm 0{,}28$ μg/g). Die Resultate bestätigten die Effektivität der Kälteeinwirkung, um gastrische Erosionen der Ratte zu verursachen. Direkter Beweis für die Freisetzung von Histamin und Serotonin aus den Schleimhautzellen während der Bildung von Erosionen wurde erreicht.

Key words: Hypothermia death, gastric erosions—Gastric erosions, hypothermia death.

Numerous mucosal haemorrhages and small ulcers in the stomach and the intestine are frequent findings in deaths due to hypothermia (Tidow, 1943; Mant, 1969; Prokop, 1965). The ulcers are obviously similar to the gastric ulcers which

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can be produced in the rat by restraint and cold exposure (Brodie and Valitski, 1963; Senay and Levine, 1967).

Simultaneously with the development of mucosal lesions, the activity of histidine decarboxylase is increased in the rat stomach during 2 hrs of cold stress at 4—7°C (Levine and Senay, 1968). Further experiments by these investigators with histidine decarboxylase and diamine oxidase inhibitors indicate the role of histamine in the formation of mucosal ulcer. This view is further supported by the observation that the injection of large doses of histamine causes gastric erosions (Franco-Brower *et al.*, 1959). In the rat, gastric mucosal histamine is stored in submucosal and mucosal mast cells as well as in basal endocrine or enterochromaffin-like cells (Thunberg, 1967).

The stomach mucosa also contains serotonin. This is stored in two types of mucosal cells: mast cells and enterochromaffin cells (Enerbäck, 1966; Hirvonen and Penttilä, 1969). It seems possible that serotonin in the gastric mucosa is affected in cold stress, since the latter condition increases the incidence of reserpine-induced mucosal lesions in the rat stomach (Reilly *et al.*, 1969). Serotonin is also an ulcerogenic agent when administered systematically (Tobe *et al.*, 1967).

The present experiments were planned with the purpose of obtaining information of the reactions occurring in the stomach in hypothermia deaths. This section of the traumatic pathology has scarcely been studied at all; animal models are therefore required to find the changes likely to occur in the cold stress. Because of the dual cellular stores of histamine and serotonin in the rat gastric mucosa, both histochemical and quantitative investigations were deemed necessary.

Material and Methods

Material

White Sprague-Dawley rats of both sexes were used. The weights varied between 195—250 g. The rats received no food for 16 hrs before the experiment but had water *ad libitum*. The animals were divided into a test group (20 rats) and a control group (20 rats).

The test rats were placed in separate plastic cages into a cold chamber with a constant temperature of —20°C and a fan which increased the cooling effect. The animals died in 3—5 hrs. When cold unconsciousness was reached, rectal temperature and cardiac function were monitored. The moment of death could be determined only by the ECG. At death, rectal temperature was 8.5—12.0°C. The control rats were also deprived of food, and they were kept at +21°C for an equal period of time. They were killed by decapitation.

The stomach was removed immediately after the death, and two samples were chilled in isopentane precooled in liquid nitrogen. Rapid quenching of the tissue is necessary to avoid diffusion of histamine from the cells.

One piece was freeze-dried at —30°C and embedded in paraffin in vacuo for the histochemical demonstration of histamine and serotonin. The second piece was used for the quantitative assays of the amines. The samples were trimmed in order to include one to two erosions. A third sample was fixed in formalin for histology.

Histology and Histochemistry

The formalin-fixed sample was sectioned at 6 μ and stained with Hematoxylin-Eosin and Cresyl violet. The latter stain is suitable for the demonstration of mucosal mast cells in formalin-fixed samples (Hirvonen and Penttilä, 1969).

The freeze-dried sample was sectioned at 10 μ, and the paraffin was removed with xylol.

Histamine was visualized with the method of Thunberg (1967). A few milligrams of ortho-phthalaldehyde (OPA) powder was placed in a jar with a cover, and the jar was preheated to vaporize the powder. The sections on slides were placed into the jar, which was kept at 100°C for 10 sec. Thereafter the sections were moved into a moisture chamber at 20°C for 5 min, and finally dried at 100°C for 10 min to stabilize the fluorescence.

The sections were viewed in a Leitz fluorescence microscope by using an excitation filter UG 1 and a barrier filter K 430. Histamine fluorescence appeared either bright yellow or deep blue.

Serotonin was visualized with the paraformaldehyde method (see Eränkö, 1967). Deparaffinized sections were kept in formaldehyde vapor at 80°C for 1 hr, and viewed in a microscope with the same fluorescence filters as those used with histamine. The colour of the fluorescence was deep yellow.

After fluorescence microscopy, the sections were fixed in 4% lead acetate and stained with 0.1% toluidine blue for the most cells. The control sections were treated in the same way with the exception that they were not kept in the aldehyde vapors.

Quantitative Assays

The sample (0.1—1.0 g) was homogenized in 4 ml of 0.4 M perchloric acid and centrifuged, and supernatant was divided into three parts: 2 ml was used for histamine extraction and 1 ml for serotonin, while 1 ml was withheld.

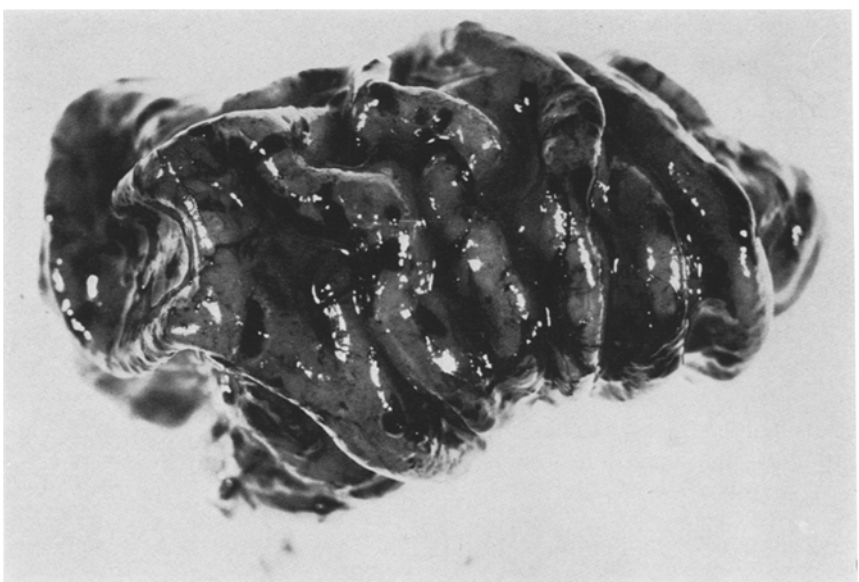

Fig. 1. Multiple erosions and haemorrhages in the glandular stomach of a rat dead from exposure to cold. Survival time three and half hours

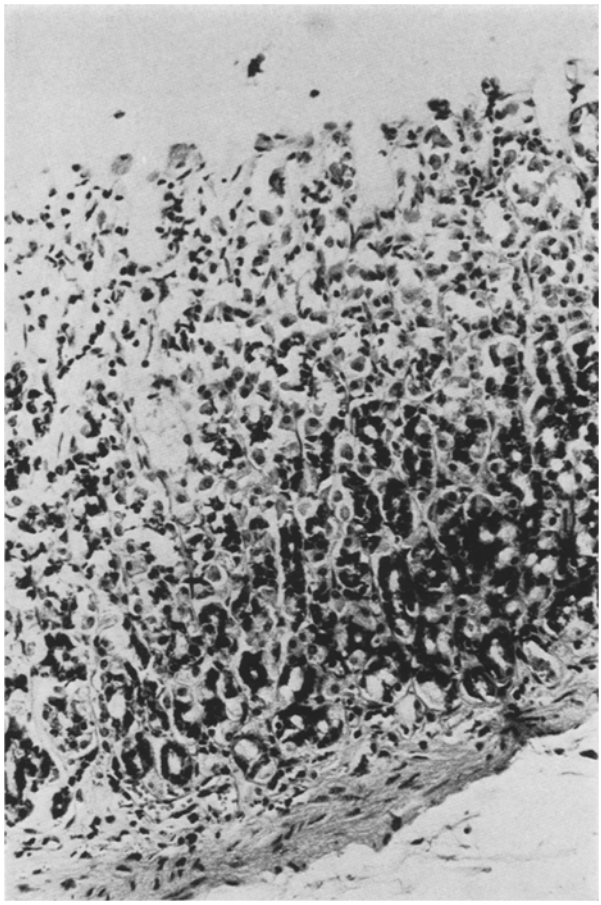

Fig. 2. Low-power view of the edge of one erosion in early phase. Superficial mucosal cells have disintegrated and many glands have suffered. Oedema is marked. Hematoxylin-Eosin. 160 ×

Histamine. The method of Anton and Sayre (1969) was slightly modified by omitting the chloroform phase.

The amount of fluorochrome formed by histamine and 0.5% OPA (Fluka) was measured with an Aminco-Bowman fluorometer. The excitation wave length used was 360 nm, emission at 440 nm (uncorrected instrument readings). The results are given as μg of free histamine per 1 g of fresh tissue.

Serotonin. This amine was extracted from the homogenate into butanol with borate buffer. The assay was performed fluorometrically with excitation at 360 nm and emission at 470 nm (uncorrected readings). The fluorochrome was developed with OPA (0.004% in 10 N HCl) by heating at 100°C for 15 min (Curzon and Green, 1970).

Results

Mucosal erosions were found in the glandular stomach of every test rat. Their number varied between 5—10, and size between 1—3 mm. Beside the erosions

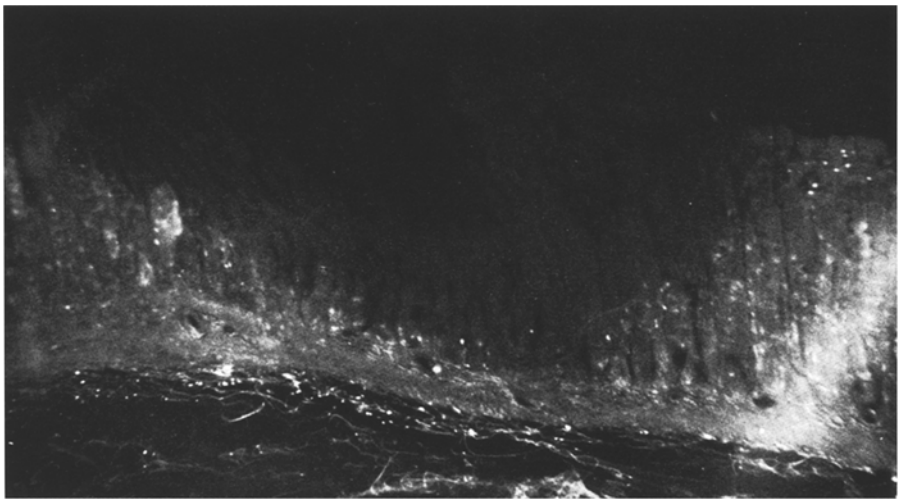

Fig. 3. Histamine fluorescence in the erosion. Fluorescence has disappeared in the erosion both from the mucosal mast cells and from the basal enterochromaffin-like cells. A normal fluorescence pattern is visible on the right. Ortho-phthaldehyde vapor treatment. 60 ×

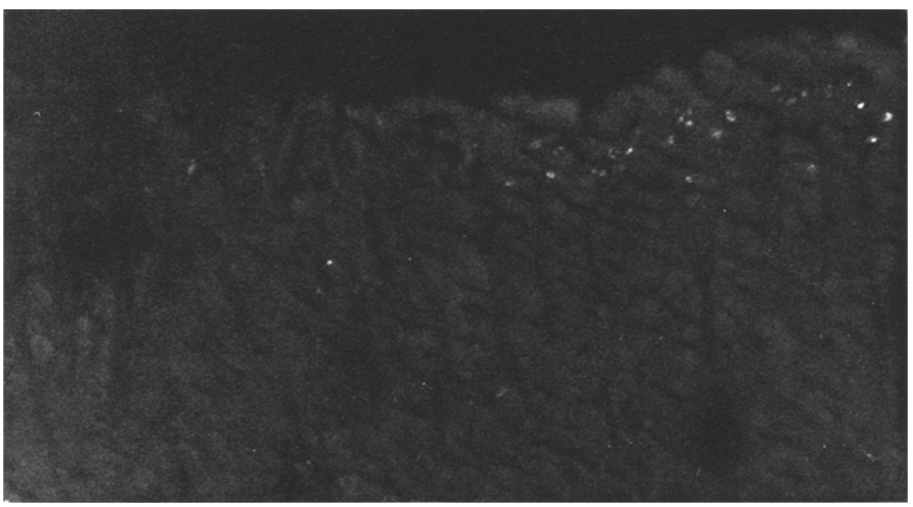

Fig. 4. Serotonin fluorescence in a small haemorrhagic lesion, i.e. in the early phase of erosion. The mucosal mast cells have lost their fluorescence in the lesion (left). Normal mucosa at right. Paraformaldehyde vapor treatment. 250 ×

large haemorrhages were also found. The lesions were mainly located in the oxyntic area, seldom in the pyloric mucosa (Fig. 1).

Histology

The erosions extended halfway through the mucosa. Oedema was prominent. Haemorrhage was not always present, but congestion of superficial vessels was evident. Most of the outer cell layers were lost (Fig. 2).

Mucosal mast cells were degranulated in the ulcer region. Partial degranulation and dispersion of granules were observed at the border of the ulcer.

Histamine fluorescence was depleted at the erosion region. This had happened both in the mucosal mast cells at the surface and in the enterochromaffin-like cells basally (Fig. 3). Submucosal mast cells had retained normal fluorescence and the normal granular pattern. Depletion of histamine from the mucosal mast cells could sometimes be observed even near the erosion in normal-looking mucosa.

Serotonin fluorescence had also disappeared from the mucosal mast cells of the erosion region (Fig. 4). In some samples serotonin was not demonstrable on large areas with no real erosions but with oedematic mucosa. The fluorescence in the submucosal mast cells was still strong, excluding an artifact. Enterochromaffin cells at the ulcer region looked intact.

Quantitative Assays

Histamine content in the control group was in average 9.89 ± 5.63 μg of histamine free base per 1 g of fresh tissue. The mean in the cold exposure group

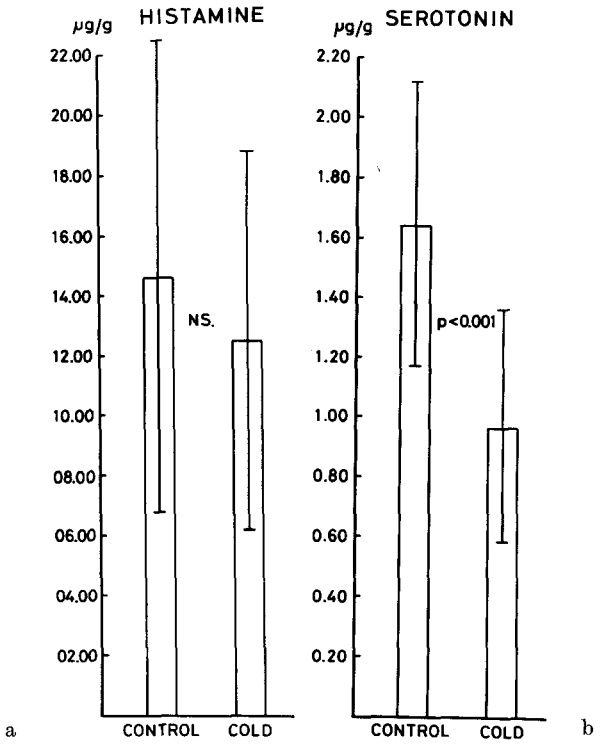

Fig. 5 a. Concentration of histamine (μg of free base per gram fresh tissue) in control stomachs and in stomachs with erosions. A slight, nonsignificant decrease was noticed in the cold-exposed rats. b Concentration of serotonin (μg of free base per gram of fresh tissue) in control stomachs and in stomachs with erosions. A significant drop of the amine was found in the cold-exposed rats

was 8.41 ± 4.69 μg/g (Fig. 5a). The decline was not statistically significant ($p < 0.15$, t-test).

Serotonin content in the control group was 0.99 ± 0.28 μg of serotonin base per 1 g of fresh tissue. The exposure group had a smaller content, the mean being 0.58 ± 0.23 μg/g (Fig. 5b). The decrease was statistically highly significant ($p < 0.001$, t-test).

Discussion

The effectiveness of severe cold stress in producing gastric erosions was again confirmed. All the cold exposed rats showed mucosal haemorrhages or large erosions at necropsy. The erosions were located on the ridges of the mucosa, and the largest of them were about 3 mm long. Microscopic investigation of the erosions showed digestion of the superficial mucosa.

The development of cold stress ulcerations is probably stimulated via three different routes. The first is cholinergic vagal stimulation, which can be eliminated with atropine, but not with central nervous depressant drugs (Rosenberg, 1967). The second route seems to be humoral stimulation of mucosal mast cells by ACTH and glucocorticoids liberated during stress. Evidence of the degranulating effect of these hormones on gastric mucosal mast cells has been found in experiments made on rats and dogs (Räsänen, 1960; Spicer and Sun, 1969). Foley and Glick (1962) were able to demonstrate a drop of mucosal histamine parallel to the degranulation of mucosal mast cells caused by corticoids. This speaks in favor of the mast cells being target cells of corticoids. The third possible mechanism is a decrease of temperature in the gastric mucosa, which is followed by cessation of blood circulation in the superficial mucosal vessels. Erosions of the mucosa are then caused by ischemia (Benjamine *et al.*, 1965).

Degranulation of mucosal mast cells occurs in gastric erosions produced by restraint of rats (Guth and Kozbur, 1969). Degranulation and loss of histamine and serotonin from the mucosal mast cells were noticed in the present cold stress erosions. The present findings confirm the assumption presented in the reports cited above that histamine and serotonin are liberated from the mucosal mast cells during the development of the lesion. Significant depletion of serotonin from the gastric mucosa was noticed in the quantitative assays, which confirmed the histochemical findings.

The mucosal serotonin is most likely to be released into blood, since increased excretion of the amine has been measured in the urine of rats during cold exposure (Le Blanc, 1963). A decreasing trend was also seen in the mucosal histamine content, but it was not significant. Histamine has also been found to increase in the rat urine in cold (Le Blanc, 1963; Feifel *et al.*, 1972). The manner histamine and serotonin are involved with the development of the erosion is not yet clear. The depletion of the amines from the mucosal cells can possibly be only a consequence of the cellular damage caused by other factors. The early liberation of the amines, however, is speaking against this assumption. For the solution of this question, experiments are needed where the cold stress is graded and the very early phase of the formation of erosions is investigated. The active role of the amines in stomach ulcerations of the rat receives support from some findings. Within the first hour of restraint stress, there occurs a marked contraction of

mucosal arterioles (Guth and Kozbur, 1969). This indicates the action of histamine and serotonin. Further, Klein *et al.* (1971) noticed degranulation of mucosal endocrine cells (enterochromaffin-like cells) in similar experiments. Disappearance of histamine fluorescence from these cells was found in the present study, which supports the former observation. The early activation of gastric histidine decarboxylase in cold stress also indicates the primary role of histamine in the rat. The role of histamine and serotonin in human gastric erosions is likely to be quite different, because storing of the amines is different.

The present findings and the reports discussed give some suggestions for developing tests for the diagnosis of hypothermia death. The assays of histamine and serotonin in the cadaver blood and the urine might prove valuable, but their usefulness requires more investigations of other animal models and necropsy cases.

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