

Histamine and Peptic Ulcer: A Prospective Study of Mucosal Histamine Concentration in Duodenal Ulcer Patients and in Control Subjects Suffering from Various Gastrointestinal Diseases*

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Histamin und Ulkuskrankheit

Zusammenfassung. In einer prospektiven Studie wurde der Histamingehalt der Korpusschleimhaut bei 100 Patienten gemessen. Diese Patienten schließen Kontrollpersonen, Ulkus-duodeni-Träger und solche mit verschiedenen gastrointestinalen Erkrankungen ein. Der Histamingehalt betrug 43 µg/g bei Kontrollpersonen und war bei Ulkus-duodeni-Kranken signifikant um 30% erniedrigt. Alle anderen Patientengruppen wiesen „normale“ Histamingehalte in der Magenschleimhaut auf. Da es bei allen Säugerarten mit Ausnahme der Ratte äußerst schwierig ist, den Histamingehalt der Magenschleimhaut durch irgendeine Maßnahme zu verändern, erscheint die Abnahme des Histamingehaltes der Korpusschleimhaut beim Ulkus duodeni besonders bemerkenswert. Unter verschiedenen Hypothesen gilt als wahrscheinlichste eine vermehrte Histaminfreisetzung beim chronischen Ulkus duodeni.

Schlüsselwörter: Histamingehalt — Magenschleimhaut — Ulkus duodeni — Gastrointestinale Erkrankungen — Histaminfreisetzung.

Summary. In a prospective study the histamine content of the mucosa of the body of the stomach was measured in 100 patients consisting of control subjects, patients with duodenal ulcer and patients suffering from various gastrointestinal diseases. The histamine content was found to be 43 µg/g in male control subjects (median) while in duodenal ulcer patients levels attained were significantly lower by about 30 per cent. In all the other groups of patients histamine concentrations in gastric mucosa were found to be “normal”. Since in most species it is extremely diffi-

cult to alter the mucosal histamine concentration by any form of treatment, the diminished histamine content of the gastric mucosa in patients with duodenal ulcer seems remarkable. Among several possible explanations offered for this finding we think the most likely is that histamine release is increased in duodenal ulcer disease.

Key words: Histamine content — Gastric mucosa — Duodenal ulcer — Gastrointestinal disease — Histamine release.

Clinical observations and experimental findings alike justify the hypothesis that nervous and humoral stimulants of gastric acid secretion are factors in the pathogenesis of chronic duodenal ulcer disease. Three basic arguments, for instance, have been advanced in favour of a role played by the vagus nerve (Dragstedt, 1962): continuous hypersecretion is observed in human duodenal ulcer disease; vagotomy abolishes the fasting hypersecretion and heals ulcers. Similar arguments can be found for a role for the family of gastrins, since continuous infusion of pentagastrin produces ulcers in cats (Konturek et al., 1973) and hypergastrinaemia in the Zollinger-Ellison syndrome is accompanied by peptic ulcer disease (Ellison and Wilson, 1964).

Histamine has long been a candidate for inclusion in this exclusive club of ulcer-promoting factors (Babkin, 1950). Thus chronic or continuous application of histamine produces ulcers in animals (Büchner et al., 1928; Walpole et al., 1940). When gastrin was isolated, however, histamine went out of fashion in gastric physiology (Johnson, 1971) and for a time it seemed that the vagus and gastrin were, together, sufficient to account for the genesis of peptic ulcers. Interest in histamine was re-awakened by the intro-

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duction of histamine H_2 -receptor antagonists by Black et al. (1972, 1973). These drugs inhibit both fasting and stimulated gastric secretion and promote the healing of peptic ulcers in man (multicentre trial, 1975). The interpretation of these findings is still disputed, however, and not necessarily has to include histamine as a physiological or pathophysiological intermediary in the stimulation of gastric secretion (Grossman and Konturek, 1973). Certainly, the arguments in favour of a role for histamine—namely that histamine induces ulcers in animals and that H_2 -receptor antagonists heal duodenal ulcers in man—appear just as cogent as the arguments advanced in favour of the gastrins and the vagus.

Certain conditions have to be satisfied, in our opinion, before histamine can be accepted as an aetiological factor in duodenal ulcer disease: (1) histamine alone or *in combination* with other gastric secretory stimulants should be shown to produce experimental ulcers, (2) in the gastric mucosa around the parietal cells, *free* histamine should occur continuously in concentrations higher than normal in order to induce fasting hypersecretion, (3) in the duodenum, *free* histamine should be present as in gastric mucosa, contributing to the pathogenesis of peptic ulcer by influencing the release of inhibitory hormones such as secretin, and by altering the microcirculation. Histamine has been shown to be capable of releasing gastrin (Fielding et al., 1974), insulin (Vance and Bragg, 1969) and pituitary hormones (Harris et al., 1952) and its actions on the microcirculation have been studied intensively (Schayer, 1961).

There are several speculative mechanisms by which free histamine levels might be elevated around the parietal cell: (1) increased plasma histamine concentrations may occur in liver insufficiency and portacaval shunting, but have been demonstrated conclusively only in acute situations such as after drug administration and operations (Lorenz et al., 1972; Lorenz et al., 1974; Lorenz, 1976), (2) histamine may be released from gastric mast cell stores in abnormally large amounts by gastric stimulants, (3) enhanced formation of (induced?) histamine may be elicited by various nervous and humoral factors (for a review see Lorenz and Pflieger, 1968), (4) finally, the inactivation of histamine may be decreased by influences on histamine methyltransferase activity (Troidl et al., 1973; Barth et al., 1975) or on histamine uptake (Lorenz et al., 1975).

At present it is not possible to measure the concentration of free histamine around human parietal cells *in vivo*. In order to investigate this problem, however, we have determined the concentration of stored histamine in human corpus mucosa, which may provide a guide to histamine release as it does in certain

“acute” situations (Kahlson et al., 1964). A prospective study was therefore performed in 100 patients at our hospital, preliminary results have already been published (Troidl et al., 1976a, 1976b).

Materials and Methods

Materials

Design and Protocols of the Study. In a prospective study biopsies of gastric mucosa for histamine estimation were taken in 100 patients from Marburg and surroundings (Nordhessen). The study ran from April 30th, 1973 till September 9th, 1973, and, at the exception of the radiological and microscopical examinations it was carried out exclusively by our working group for gastric disease. This group for the purposes of the study, consisted of two clinical surgeons, two theoretical surgeons (for definition see Lorenz et al., 1976a), four technicians and one medical student.

The various parts of the study (Table 1) were performed in a special suite of rooms, at definite times and always by the same investigators and technicians.

(1) Photostat copies were made of the patients' case histories, which had been completed strictly from data provided by the family doctors and by other hospitals where the patients had previously been treated. In this way no information was lost during the routine work at our clinic. In three cases only earlier findings in other hospitals provided the diagnosis, because due to spontaneous healing of duodenal ulcers the lesions could no longer be demonstrated at our hospital. The clinical questionnaire was completed at our follow-up clinic (Rohde et al., 1976a).

(2) Fibre-gastroscopy was performed by the two clinical surgeons in the morning on Monday and Wednesday each week.

Table 1. Design and protocols of the prospective study on mucosal histamine concentration in various gastric diseases

1. Findings obtained by
— case history (completed by data from other hospitals)
— clinical questionnaire with 60 questions
— oesophago-gastro-duodenoscopy
— microscopical examination of biopsy specimens from oesophagus, stomach and duodenum
— radiology of stomach, intestine and biliary tract
— gastric secretory tests
— operative findings in all cases submitted to surgery
2. Diagnoses established by
— preliminary decision made by two clinical surgeons and by one theoretical surgeon independently of each other
— final decision made by a panel discussion of the three investigators
3. Control subjects and patients classified by
— present diagnosis
— accompanying diseases
— earlier history
4. Parameters investigated in the study
— Histamine concentration of corpus mucosa
— Basal secretion and maximum secretory response to pentagastrin: ^a Volume acidity, acid output, pepsin output

^a The results on these parameters will be published in forthcoming papers

All patients submitted to this procedure for diagnostic purposes (none under emergency conditions) were included, without any special selection of cases. By design, also patients with insignificant, but undiagnosed, abdominal complaints were included in the trial so that we might obtain control subjects. The decision in favour of endoscopy, however, in these patients was made by physicians not involved in this study (they belonged to other sections of our clinic or to other departments). There was no selection of subjects according to the day of the week, so as to have, for example more endoscopies on duodenal ulcer patients on Monday and more in other groups of patients on Wednesday. Hence members of the different groups of patients were likely to have been distributed randomly in our sample, they could so be used as controls for the duodenal ulcer patients because they were subjected to the same physical and psychological stresses. The criterion used to include subjects in the trial was the performance of endoscopy, since in each of a consecutive series of 100 patients submitted to this procedure, biopsy specimens were taken for the determination of mucosal histamine concentration.

(3) Microscopical examination of the biopsy specimens was performed at the Department of Pathology, Marburg, and radiology at a division of our Department of Surgery following a barium meal and administration of iodine benzamic acid (Osbil®, Byk-Gulden, Konstanz).

(4) According to the procedure shown in Table 1, the diagnoses were established separately by each of the three investigators.

(5) Following the final decision reached during a panel discussion, the patients were integrated into one of several groups of patients (Table 2) on the basis of the present diagnosis, the accompanying diseases and the diseases found earlier in the case history. Their earlier case history sometimes led to considerable problems in classifying them as "control" or "duodenal ulcer patient". A gastrointestinal disease in earlier life for example might have caused biochemical or morphological alterations in the gastric mucosa which may have continued to exist over the years and influenced the histamine content of the gastric mucosa in this study.

For this and other reasons, 17 subjects were excluded from the study by an escape clause because: (1) they could not be reliably assigned to one of the groups in Table 2; (2) several diseases co-existed and none could be definitely identified as the main disease; (3) the case history was incomplete (foreign worker); (4)

patients had been submitted to gastric surgery before the start of the trial. Details of each of cases excluded are given in Table 3.

To avoid the introduction of bias the results of the histamine estimations were not recorded until the patients became part of the one or the other group shown in Table 2.

Definitions of Control Subjects and Patients (Table 2)

Control Subjects. These patients were considered as "healthy" with respect to their upper gastrointestinal tract. (1) They were submitted to endoscopy for investigation of non-specific abdominal complaints. (2) Disease of the oesophagus, stomach and duodenal bulb could be excluded by endoscopy and microscopical examination of biopsy specimens. (3) Other diagnostic procedures, such as radiology, clinical investigation and clinical-chemical tests also did not reveal any disease of the upper gastro-intestinal tract like significant hiatal hernia (see below), cholecystitis or pancreatitis. (4) The time-course of their disease excluded an upper gastro-intestinal illness, but like other groups of patients in our study the control subjects fully underwent the emotional stress of the hospital atmosphere. (5) The final diagnoses in the control subjects were: insignificant hiatal hernia (10), constipation (5), dietary indiscretion (3), "nervous stomach" (3), adhesions after appendectomy (3), diseases of rectum (3) [2 carcinomas, 1 haemorrhoids], aorto-femoral bypass (1), sigmoid stenosis (1), umbilical hernia (1), possible trauma of the intestine (1).

Duodenal Ulcer Patients. The diagnosis of duodenal ulceration was established in each patient, usually by all the procedures listed in Table 1, and at least by endoscopic, radiological and histological examination. Accompanying diseases of the upper gastrointestinal tract were excluded but the presence of small and insignificant hiatal hernias was ignored for purposes of classification.

Gastric Ulcer Patients. In all cases the diagnosis was established not only by endoscopy, radiology and histology but also by operation and intra-operative frozen section microscopical examination (to exclude carcinoma!).

Hiatal Hernia Patients. Hiatal hernia of insignificant degree are considered as an anomaly frequently occurring with more or less

Table 2. Attributes of "control subjects" and patients included in the trial. The ages of female control subjects ranged from 24 to 76 years. 5 patients who were not operated upon and 12 following gastric surgery were not included and are described in Table 3

Diagnoses	Number of subjects investigated	Sex		Age (Range) (years)	Weight (Range) (kg)
		Male	Female		
Control subjects	31	21	10	17-81	69-110
Duodenal ulcer	18	17	1	19-74	47-96
Gastric ulcer	10	9	1	39-87	47-61
Hiatal hernia	6	2	4	53-84	57-93
Gastric carcinoma	5	5	0	43-78	48-75
Cholecystitis	4	0	4	35-60	65-80
Gastric erosions	3	2	1	25-66	52-84
Zollinger-Ellison Syndrome	1	1	0	36	50
Atrophic gastritis	1	0	1	72	52
Pancreatitis	1	1	0	17	95
Oesophageal varices due to portal vein cavernoma	1	1	0	27	65
Alimentary obesity	2	2	0	24; 32	144; 155
Total	83	61	22	17-87	47-155

Table 3. Attributes and mucosal histamine concentrations of patients excluded from the trial by escape clause. ♂=male; ♀=female, — means "no significant disease". Concerning the criteria for exclusion, see text in section 1 of Materials and Methods. Vagotomy=selective gastric vagotomy and pyloroplasty, B I or B II=Billroth I or II enteroanastomosis, 2/3-resection and antrectomy, all operations as described by Seidel et al. (1973), patients after operation came to our follow-up clinic

Name	Sex	Age (years)	Weight (kg)	Diagnoses			Mucosal histamine content (µg/g)
				present	accompanying	earlier	
Patients not operated (escape criteria 1–3)							
B.F.	♂	39	65	Scar in duodenal bulb	Unknown	Unknown	30.4
B.P.	♂	43	72	Chronic liver disease	Atrophic gastritis	—	14.4
Schw.W.	♂	43	68	Oesophagitis ulcerosa	Pancreatitis	—	28.7
Sch.H.	♂	36	91	Intrapyloric ulcer	—	Cholecystitis	78.7
W.H.	♂	17	95	Duodenal ulcer	Pancreatitis, alcoholism	—	40.3
Patients having undergone gastric surgery (escape criterion 4)							
E.K.	♂	35	87	Vagotomy	—	Duodenal ulcer	63.5
K.K.	♂	41	62	Vagotomy	—	Duodenal ulcer	51.6
K.M.	♂	67	56	Vagotomy	—	Duodenal ulcer	47.9
R.H.	♂	35	72	Vagotomy	cholecystitis	Duodenal ulcer	102.0
Sch.J.	♂	68	74	Vagotomy	—	Duodenal ulcer	49.7
T.A.	♂	67	69	Vagotomy	—	Duodenal ulcer	68.8
P.E.	♂	43	75	Recurrent ulcer (vagotomy)	—	Duodenal ulcer	35.0
C.M.	♂	47	50	Vagotomy + antrectomy (B II)	—	Duodenal ulcer	70.7
T.E.	♂	52	62	2/3-resection (B I)	—	Gastric ulcer	63.5
G.J.	♂	48	63	Reoperation (B II)	—	Jejunal ulcer	9.8
N.A.	♂	71	56	2/3-resection (B II)	Cholecystectomy	Gastric ulcer	57.0
Sch.R.	♀	45	72	2/3-resection (B II)	—	Duodenal ulcer	41.1
Total: 17 patients	16 ♂, 1 ♀	17–71	50–95	12 operated, 7 vagotomies, 5 gastric resections	4 patients with significant accompanying diseases	Operations mainly for duodenal ulcer	9.8–102.0

remarkable complaints (Salter, 1975). Thus this diagnosis was only accepted for classification in patients if it was thought very likely to be the dominant reason for the abdominal complaints. The complaints, especially during the night (Earlam, 1976), had to be so intense as to render treatment mandatory. The diagnosis of hiatal hernia was made using data from the case history, clinical examination, endoscopy and radiology (Salter, 1975).

Other Groups of Patients. The diagnosis and classification were accomplished by the method outlined in Table 1, as well as by common clinical-chemical tests. The diagnosis of Zollinger-Ellison syndrome was established by the finding of elevated basal serum gastrin levels on 6 occasions, increase in serum gastrin levels following secretin injection and calcium infusion, and of elevated basal serum gastrin levels following total gastrectomy (radioimmunoassay of gastrin was performed by R. Arnold, M.D., Department of Internal Medicine, University of Göttingen).

Preparation of Biopsy Specimens. The panendoscope 7089 P from ACMI (Wappler, Munich) was used, with straight optics and a biopsy forceps with a spike (2.9 mm), in a modification of type 7035 A. The jaws of the forceps were longer (3.9 mm) and deeper (0.9 mm) than the original type so that larger biopsies could be obtained. Four biopsy specimens were taken from the body mucosa of each of the subjects. Three of them were used for mucosal histamine determination and one for microscopical examination. The samples for histamine assay were weighed immediately after withdrawal on a microbalance (Sartorius, Type 2774) and

suspended each in 4 ml 0.4 M HClO₄. Until histamine determination these mixtures were kept at –20°C in a deep-freezer for no longer than two weeks.

Drugs and Reagents. Before endoscopy, the patients drank 1–2 ml SAB-Simplex® (Parke-Davis) dissolved in 50 ml tap water (0.6 ml SAB-Simplex® contained 40 mg dimethylpolysiloxane, 0.1 mg sodium saccharin, 1.2 mg sodium cyclamate). As premedication 15 min before starting endoscopy, the patients were given i.v. atropine (0.01 mg/kg), hyoscine-N-butylbromide (0.3 mg/kg), trifluoromazine hydrochloride (0.15 mg/kg) and diazepam (0.1 mg/kg). None of these drugs caused significant histamine release which might have lowered gastric histamine concentration (Lorenz et al., 1974). Only the substances described by Lorenz et al. (1972a, 1974a) were used, as reagents in order to exclude impurities interfering with the assay. Twice-distilled water was prepared in a quartz still (Köttermann Ltd.).

Methods

Fluorometric Assay of Histamine in Biopsy Specimens

Histamine was determined according to a modification of our Dowex-method (Lorenz et al., 1970). The modification was necessary in order to increase the sensitivity of the assay for measuring the amine only in a few milligrams of tissue (Lorenz et al., 1975). The perchloric acid mixtures containing the biopsy specimens were homogenised with an Ultraturrax homogenizer using a microshaft TP 10 N. The total homogenate which showed a consistency of

turbid water was applied to the Dowex columns without prior centrifugation but only after adjustment to pH 6.5 by 5 M NaOH and 0.1 M sodium phosphate buffer (pH 6.5). After elution of histamine from the columns, condensation with 0.1% o-phthaldialdehyde was carried out to lower the reagent blanks and to increase the sensitivity of the assay (Lorenz et al., 1976b). Thanks to a new photomultiplier used (Lorenz et al., 1974a), very small slide widths could be applied on the Zeiss fluorometer. Histamine was expressed as μg histamine dihydrochloride/g.

Miscellaneous Techniques to Ensure Precision and Accuracy of the Histamine Determination. A fourth sample was obtained for microscopical examination from the area where the three biopsy specimens were taken for histamine assay. This enabled to assess the depth of the mucosal sample and to establish the pathological diagnosis. The techniques involved are described by Rohde et al. (1976b). The precision and accuracy of the histamine determination were tested both within the same day and from day to day (quality control). The results of these studies were published in detail elsewhere could be applied on the Zeiss fluorometer. Histamine was expressed as μg histamine dihydrochloride/g.

Statistical Methods. Since the frequency distribution of the mucosal histamine contents in duodenal ulcer patients diverged considerably from normal, the median-percentile system was used for parameters of location and variance. Significance was tested by means of the Mann-Whitney-U test, which is suitable for non-parametric data (Sachs, 1974).

Results

1. Histamine Concentrations in Body Mucosa of Control Subjects

In 31 control subjects, the histamine content of the body mucosa was 40.7 (31.4–46.1) $\mu\text{g/g}$ fresh weight (median, 1st–3rd quartile). This is a very high his-

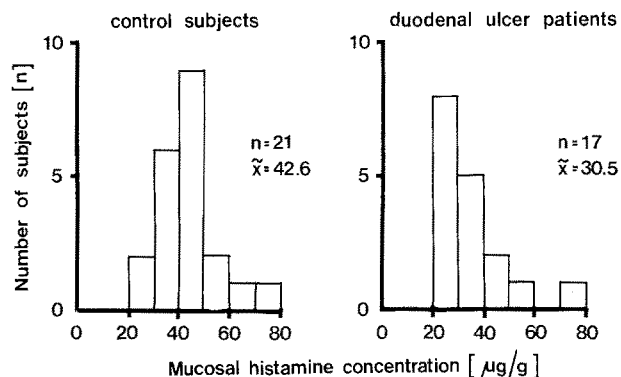


Fig. 1. Histograms of histamine concentrations in human corpus mucosa of control subjects and duodenal ulcer patients. Only male subjects were included. \bar{x} =median. Statistical significance (Mann-Whitney test) between the two groups of individuals $p < 0.025$

mine concentration compared to that of other human tissues (Lorenz et al., 1973). The values were fairly normally distributed, as shown in Figure 1 for male subjects, but the same was true for the whole sample of "normal" subjects. There existed no obvious correlation between histamine levels and age, though the small number of subjects in the different groups may not have been sufficient for reliable calculation of statistical significance (Table 4).

In male control subjects the mucosal histamine content attained 42.6 (31.4–49.6) $\mu\text{g/g}$ ($n=21$) and in female controls 35.8 (24.5–46.3) $\mu\text{g/g}$ ($n=10$). The difference of 16% between males and females was not statistically significant.

Table 4. Histamine concentrations in human corpus mucosa of control subjects and duodenal ulcer patients depending on age. Only male subjects were included. Statistical significance (Mann-Whitney test) between the two groups of individuals: * $p < 0.1$, ** $p < 0.2$

Diagnosis	n	Histamine content ($\mu\text{g/g}$)					
		< 20	20–34	35–49	50–64	65–79	> 79 years
Control subjects	1	33.5	22.7	23.2	35.6	31.8	43.3
	2		30.3	31.4	41.1	61.0	
	3		40.9	37.8		76.8	
	4		44.4	40.7			
	5		45.7	49.5			
	6		45.9	55.6			
	7		46.3				
	8		51.2				
Median	—	—	45.1*	39.3**	38.3	61.0	—
Patients with duodenal ulcer	1	71.5	21.7	22.3	24.0	26.7	—
	2		24.5	23.1	30.5		
	3		37.6	25.0	33.8		
	4		38.1	26.3			
	5		42.5	29.8			
	6			47.7			
	7			51.4			
Median	—	—	37.6	26.3	30.5	—	—

2. Histamine Concentrations in Corpus Mucosa of Patients with Chronic Duodenal Ulcer

In this group of patients values can be presented only for male subjects, since there was only one woman. The mucosal histamine content in 17 males was 30.5 (24.0–42.5) $\mu\text{g/g}$ (median, 1st–3rd quartile). The frequency distribution of the histamine values, however, was far from normal (Fig. 1) and corresponded more to a log-normal distribution. By the Mann-Whitney test, the mucosal histamine concentration in duodenal ulcer patients was found to be significantly by 30% lower ($p < 0.025$) than in male control subjects (Fig. 1).

As in control subjects there was no detectable correlation between mucosal histamine content and age in duodenal ulcer patients (Table 4). It seemed remarkable however that these two groups of individuals were really well matched for age. The difference in the mucosal histamine content between the two groups of patients could be demonstrated in all age groups which contained more than one individual (Table 4). Since the two groups of subjects were also well matched for weight, it seems fair to conclude that the difference in mucosal histamine concentration between "normal" control subjects and duodenal ulcer patients was well established.

The only female duodenal ulcer patient in our trial also had a prepyloric gastric ulcer (Dragstedt combination). Her mucosal histamine content was relatively high (81 $\mu\text{g/g}$). No conclusions can be drawn since we have no further experience of mucosal histamine levels in this disease in women.

3. Histamine Concentrations in Corpus Mucosa of Patients with Various Gastric Diseases

With the exception of one patient suffering from a Zollinger-Ellison syndrome, all other groups of patients showed mucosal histamine concentrations higher than those found in duodenal ulcer patients (Table 5).

For direct comparison with the values in other groups of patients, the histamine concentrations in the 9 male subjects with gastric ulcer should be noted: 33.8 (32.8–47.4) $\mu\text{g/g}$ (median, 1st–3rd quartile). The only female subject with a gastric ulcer showed a mucosal histamine concentration of 28.8 $\mu\text{g/g}$.

Patients with significant hiatal hernia were not different from "normal" control subjects with respect to their mucosal histamine content. Patients with cholecystitis (females only) had slightly higher values. Patients with gastric cancer (male subjects only) had slightly lower values than those found in the control

Table 5. Histamine concentrations in human corpus mucosa of patients suffering from various gastro-intestinal diseases. Male and female subjects were included. Due to the small number of individuals in the groups only for histamine values of male gastric ulcer patients statistical significance could be calculated in comparison to control subjects and duodenal ulcer patients. Using the Mann-Whitney test in none of the two cases the differences were significant which should not be expected from the small number of patients (cf. those for control subjects and duodenal ulcer patients)

Diagnosis	n	Histamine content ($\mu\text{g/g}$)	
		Median or single values	Range
Gastric ulcer	10	37.2	15.5–98.8
Hiatal hernia	6	37.2	13.4–66.8
Gastric carcinoma	5	38.3	20.8–70.7
Cholecystitis	4	48.0	31.7–71.0
Gastric erosions	3	43.0	—
Z.E. syndrome	1	13.3	—
Atrophic gastritis	1	95.8	—
Pancreatitis	1	40.3	—
Oesophageal varices	1	63.0	—
Obesity	2	40.9, 45.7	—

subjects (Table 5). The single cases of Zollinger-Ellison syndrome and of atrophic gastritis which have widely differing gastric histamine levels, do not permit to draw a definite conclusion and further investigation is needed.

Finally, in the patients of the "escape" group (Table 3) histamine concentrations were in generally considerably higher than those in duodenal ulcer patients. Mucosal histamine contents after vagotomy, in particular were found to be even higher than those observed in "normal" control subjects. This interesting result has been confirmed in a second prospective study to be published elsewhere in greater detail (Troidl et al., 1976b).

Discussion

1. Reliability of the Findings on Mucosal Histamine Concentrations in Various Groups of Patients with Gastrointestinal Disease

There were three main problems which influenced the reliability of our results on mucosal histamine concentration: reliability of the histamine assay in biopsy specimens, relative thickness of mucosal biopsies and reliability of allotting patients to the various groups.

Using a modification of the fluorometric method for histamine determination in tissues (Lorenz et al., 1970), this amine could be determined in all gastric biopsy specimens investigated (Lorenz et al., 1975;

Lorenz et al., 1976b). The specificity of the assay has been assured by the usual tests of identity (Lorenz, 1975; Lorenz et al., 1976), while the precision and relative accuracy of this technique have been shown by carrying out quality control experiments within the day and from day to day (Lorenz et al., 1975; Rohde et al., 1976b). There is thus no doubt about the reliability of the histamine assay in biopsy specimens.

As shown by our group (Rohde et al., 1976b) and others (Heinkel et al., 1960; Kobayashi et al., 1974) the relative thickness of mucosal particles obtained by biopsy varies considerably. Only in 18% of the specimens was the total thickness of the mucosa reached. In 55%, two-thirds, and in 27%, one-third of the body mucosa were obtained. This problem however seemed to be solved by taking three biopsy specimens from each of the subjects, so minimising the possibility that only superficial parts of the gastric mucosa were taken, since the relative thickness of mucosal particles was randomly distributed in the same patients. Furthermore, histamine and mast cells containing histamine (see below) have been found to be fairly uniformly distributed throughout the thickness of the mucosa (Murray and Wyllie, 1964; Håkanson et al., 1970) or to have some degree of preference for the more superficial layers of the body mucosa (Smith, 1959; Räsänen, 1958).

The third problem, i.e., establishing the diagnoses and to group patients into the various groups with sufficient accuracy, represented the greatest source of difficulty in our trial. Initially it seemed very simple to define and to establish the diagnosis of a duodenal ulcer, but for a variety of reasons it proved to be difficult in individual cases (Troidl et al., 1975). Similar comments have been made by Goligher et al. (1972). In addition the detection of accompanying diseases and a critical evaluation of their significance was very laborious and always contained an element of subjectivity and personal bias. The previous history of a patient sometimes contained conflicting information which made it difficult to include him in a distinct group in our trial. For example, can a patient be considered to be a "normal" control subject if he has had significant "gastritis" for a relatively long period several years ago? In our panel discussion a new dimension was introduced by the realisation that, aside from other attributes, human beings differ from guinea-pigs mainly by their personal histories! On account of all these difficulties it was important that the values for the mucosal histamine concentrations should be added to the other data of each individual patient only after the final decision had been made about his belonging to one or another group in our trial.

2. Histamine Concentrations in Human Gastric Mucosa: Alteration in Duodenal Ulcer Disease and Hypotheses about its Cause and Clinical Significance

In this investigation mucosal histamine concentrations were determined in a relatively large number of human subjects. Samples of gastric mucosa were taken under more favourable conditions (i.e. closer to normal physiological conditions) than has been the case in all other previous studies. This enabled us to conclude at a relatively high reliability that the histamine content in "normal" human gastric mucosa was indeed very high and that it was significantly different from normal in duodenal ulcer disease. Since in any species except the rat (Kahlson et al., 1964) it is extremely difficult to alter the mucosal histamine concentration by any treatment (cf. Lorenz and Pfeleger, 1968) the decrease in histamine content in duodenal ulcer disease seems to be remarkable.

To our knowledge, histamine in human gastric mucosa is localized exclusively in mast cells. Studies using fluorescent microscopy have shown that cells in this tissue which emit luminescence after treatment with o-phthalaldehyde were always mast cells if the sections were consecutively stained with toluidine blue (Håkanson et al., 1969). These observations were in contrast to the findings of Tobe and Tanaka (1972) who described histamine-induced fluorescence coming from parietal cells they did not exclude the possibility that the fluorescence which they observed originated from mast cells closely related to parietal cells (toluidine blue staining was not done!) (Lorenz et al., 1971). According to Räsänen (1958) the highest density of mast cells in human corpus mucosa is indeed found in the layers which also show the highest density of parietal cells. His finding that there is a lower density of "detectable" mast cells in duodenal ulcer patients than in normal subjects provides support for our results (Räsänen, 1958).

Histamine in mast cells is *stored* histamine which has been predominantly measured in our study. The finding of a decrease in stored histamine in duodenal ulcer disease first led to the hypothesis that this might be due to an *augmented histamine liberation* in this disease. In short-term experiments indeed a decrease of tissue histamine content reflects histamine release, mast cell degranulation and occurrence of the amine in body fluids (Lorenz et al., 1973a; Lorenz et al., 1974b). Similar results were obtained from rat gastric mucosa where histamine is not secreted from mast cells but from enterochromaffin-like cells (Kahlson et al., 1964; Thunberg, 1967; Håkanson et al., 1967; Lundell, 1974). Since duodenal ulcer disease is a chro-

nic long-lasting process, our hypothesis is not (entirely) convincing, but nevertheless remains as a possible explanation for our findings.

There is good evidence that duodenal ulcer disease is associated with increased vagal activity (Dragstedt, 1962). In rats vagally stimulated gastric secretion is accompanied by histamine release from gastric mucosa (Kahlson et al., 1967; Thunberg, 1967). Although the bulk of this histamine may be derived from enterochromaffin-like cells, a part of it may also be liberated from the "atypical" mast cell in the more superficial layers of gastric mucosa (Thunberg, 1967; Aures et al., 1968), because vagally stimulated gastric secretion is indeed inhibited after degranulation of mucosal mast cells in rats (Hirvonen et al., 1967). Mast cell degranulation furthermore is observed in experimental gastro-duodenal ulcers produced by stress in rats (Guth and Hall, 1968; Guth and Kozbur, 1968, 1969) and this process is largely blocked by vagotomy. None of these arguments provides clear evidence of vagally stimulated histamine release in human subjects—in particular, in duodenal ulcer patients—but they do not exclude the possibility that our hypothesis of an augmented histamine release in duodenal ulcer disease might be correct. It would then be expected that the mucosal histamine content of duodenal ulcer patients increase after vagotomy, which does in fact happen (Troidl et al., 1975a, 1975b). This finding increases the chances that our hypothesis is correct.

A second hypothesis to explain our findings is that of *decreased histamine formation* in duodenal ulcer disease. It is difficult to accept this idea at present for the following reasons. Firstly, it is by no means accepted that histamine be formed in human gastric mucosa (Lorenz et al., 1976c). The existence of a specific histidine decarboxylase in human gastric mucosa has been both accepted and denied. The existence of a non-specific histidine decarboxylase has been shown by all investigators so far but its physiological significance has been frequently questioned (for a review of the arguments, see Lorenz et al., 1976c). Secondly, the only species which possesses a high activity of specific histidine decarboxylase in gastric mucosa is the rat. In that species vagal stimulation does not depress histamine formation but enhances histidine decarboxylase activity (Kahlson et al., 1967; Thunberg, 1967). Thus the hypothesis seems unconvincing that histamine formation is decreased in duodenal ulcer disease.

A third hypothesis is that there exists an *increased inactivation of gastric histamine* in duodenal ulcer disease. Barth et al. (1977) however found that histamine methyltransferase activity—the only enzyme at present known to inactivate histamine in human gastric mucosa—is significantly diminished in the corpus mucosa

of duodenal ulcer patients. Thus this hypothesis also lacks conviction.

The effects of histamine H_2 -receptor antagonists, metiamide and cimetidine on healing duodenal ulcers (multicentre study, 1975) could be explained by two different mechanisms, namely in that (1) it prevents abnormally large amounts of histamine to be released at the H_2 -receptors of the parietal cells. (2) or that it activates histamine methyltransferase in gastric mucosa which, in turn, leads to faster inactivation of the released histamine. In vitro this effect was shown by Barth et al. (1973, 1973a). In vivo there are findings which support this hypothesis (Thjodleifsson and Wormsley, 1975).

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