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1. DISTRIBUTION AND METABOLISM OF HISTAMINE

Histamine content, diamine oxidase activity and histamine methyltransferase activity in human tissues: fact or fictions?[1]

R. HESTERBERG*, J. SATTLER†, W. LORENZ†, C.-D. STAHLKNECHT*, H. BARTH‡, M. CROMBACH† and D. WEBER†[2]

*Surgical Clinic, Centre of Operative Medicine I; †Department of Theoretical Surgery, Centre of Operative Medicine I, University of Marburg/Lahn, Robert-Koch-Str. 8, D-3550 Marburg, FRG; ‡Centre of Research, Grünenthal GmbH, Zieglerstr. 6, D-5100 Aachen, FRG

Abstract

To understand the role of histamine in the aetiology and pathogenesis of human diseases reliable data are urgently needed for the histamine content and for the activities of histamine-forming and -inactivating enzymes in human tissues. In order to make a substantial progress toward this aim a tissue-sampling programme during surgical interventions was carefully conceived and conducted. From March 1982 until January 1983 106 tissue specimens were taken from 56 patients who underwent surgery. Only healthy tissues, not injured or oedematous, and without adherent structures were taken by only one surgeon who was interested in this research and experienced in tissue preparation procedures in biochemistry. The times of 'warm' ischaemia during the operative procedures were visually estimated, the times between resection of the organs or specimens and deep-freezing of the tissues were precisely recorded.

Compared to previous work in the literature and especially to our own work using the same assays for determination higher histamine contents were found in this study in most of the tissues, in particular in the gastrointestinal tract. Also the diamine oxidase activities were considerably higher in many organs, e.g. 3–4 times higher in the gastrointestinal tract when compared with those in publications of our group who used always the same analytical test. However, the histamine methyltransferase activities in this study were not at variance to those determined in previous investigations. Many of them were reported in this communication for the first time.

Since the methods for histamine determination and those for measuring enzymic activities were not different in this study and in previous communications of our group we are convinced that the optimized tissue-sampling and -preparation techniques were responsible for the higher values in this communication. But the problem of the 'warm' ischaemia period could not be solved by sample-taking procedures of this type during operations. There are good reasons to prefer biopsy specimens for the analysis of histamine storage and metabolism in human tissues in health and disease, but – unfortunately – they are not always available.

Introduction

In human tissues histamine content and diamine oxidase (DAO) and histamine methyltransferase (HMT) activities were altered in several common diseases.

An increase of histamine storage was found in liver and skin in biliary diseases [1] and thyreoid disorders [2], respectively. The histamine content was lowered in the gastric corpus mucosa of patients with peptic ulcer [3, 3a, 4]. In subjects with hypernephroid carcinoma the histamine concentration was very low in the tumour itself, but very high in the close vicinity of the malignoma [5].

Diamine oxidase activity (diamine: oxygen oxidoreductase (deaminating) (EC 1.4.3.6)) was decreased in the tumour tissue of colonic cancer [6], but increased in that of gastric cancer [7] and in particular in the adjacent mucosa [6, 7]. In small cell bronchial cancer, however, the opposite finding was obtained: the enzymic activity was increased in the tumour itself when compared with that of the normal lung tissue [8].

Finally, also alterations of histamine methyltransferase activity (*S*-adenosyl-L-methionine: histamine *N*-methyltransferase (EC 2.1.1.8)) were detected in human diseases. The activity of the enzyme was significantly decreased in the gastric corpus mucosa of patients with duodenal ulcer [9, 4].

Before, however, hypotheses and advanced conclusions can be drawn from all these observa-

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tions with regard to an aetiological or pathogenetic role of histamine in these diseases or in complications of these diseases (e.g. haematuria or recurrent ulcer) the data have to be critically analysed for their reliability. For instance in patients with duodenal ulcer histamine determinations in the oxyntic mucosa led to completely different results in two clinical studies [3, 10]. TROIDL et al. [3] found a decrease of the histamine content by 30% in duodenal ulcer patients compared to that of healthy controls whereas DOMSCHKE et al. [10] observed an increase by 80%. However, in these two studies the values for the control groups were at variance by one order of magnitude. In a discussion between the two groups of investigators it became apparent that the very low histamine concentrations measured by DOMSCHKE et al. [3] were not the result of the high specificity of the radioenzymatic assay, but of the long period of ischaemia after sample-taking. When this time was substantially shortened the same histamine concentrations in the gastric mucosa were determined [11] as reported by TROIDL et al. [3]. Unfortunately in most reports on histamine storage and metabolism in human tissues the time intervals and techniques for tissue sampling are not described precisely and extensively enough. Thus differences in the results on tissue histamine contents such as those in gastric ulcer cannot be elucidated with satisfactory certainty.

For this reason a tissue sampling programme for measuring histamine content and activities of histamine-inactivating enzymes in human tissues was carefully planned and conducted. Surgical specimens were used since in a series of organs it is not possible to obtain biopsy specimens from patients or healthy human volunteers for this kind of biochemical analysis.

Materials and methods

Materials

Frame of the study and patients. Tissue specimens for histamine assays and determination of DAO and HMT activities were taken from 106 organ preparations of 56 patients in the operating theatres of the Surgical Clinic from March 1982 until January 1983. On the average ($\bar{x}$) the age of the subjects was 59 years (range 21–77), 32 were male and 24 female, and the general characteristics of the patients, their diseases and operating treatment which may influence the results on histamine content and enzymic activities were compiled in Table 1. The patients were anaesthetized with hexobarbital sodium or etomidate, fentanyl, suxamethonium-alcuronium and halothane-nitrous oxide. They were operated by 12 surgeons who were on consultant to senior registrar level of their surgical training. However, all tissue samples were taken and prepared by only one surgeon (R.H.) who was interested in this research and experienced in tissue preparation procedures for biochemical studies [6]. The selection of patients depended entirely on his presence in the operating theatre and on the feasibility to accomplish all other criteria (see below) for accurate sample-taking. By this technique for recruitment of subjects at least an element for unbiased selection was introduced in the study. Only in the last two months toward the end of the trial the tissue groups of heart and pancreas were voluntarily filled up with cases.

Before starting the tissue collection the following criteria for sample-taking were defined: (1) The tissue specimen should only be dissected from macroscopically healthy areas of the surgical preparation, not infarcted with blood, oedematous or crushed and not contaminated by adherent structures such as connective tissue or fat. (2) Ischaemia was denoted as 'warm' ischaemia and 'cold' ischaemia. Warm ischaemia was the time interval between ligation of the blood vessels or starting resection until the removal of the surgical preparation, cold ischaemia was the time between removal of the organ and deep-freezing of the sample at −40°C. (3) For special organs additional criteria had to be fulfilled. From lung only parenchyma was taken and bronchial tubes were dissected as far as possible, from liver and spleen wedge-shaped excisions were used from the frontal margins of the organ, from stomach and gut only mucosa was used which was not scraped off, but carefully dissected. Heart tissue was obtained from the papillary muscle of the left ventricle.

The tissue samples after preparation had a weight of 2 – 3 g except the heart specimens which were available only in 0·5–1 g quantities. Before freezing they were rapidly and at random divided into 3 parts for histamine assays and tests of DAO and HMT activity and placed in a Petri dish at the 3 corners of a triangle. The position 1–3 was marked and the specimen 1 always used for histamine, specimen 2 for DAO and specimen 3 for HMT. The samples were stored up to 4 weeks at −40°C, histamine was always determined first and DAO activity second by the same technician whereas HMT activity was measured last by a second investigator, usually a week later than histamine content and DAO activity. Care was taken that the sample for HMT activity was not thawed during the determinations of histamine content and DAO activity.

Chemicals

For histamine assays the same substances and solvents were used as described by LORENZ et al. [11a], for measuring DAO activity those reported by KUSCHE et al. [13] and for testing HMT activity those described by BARTH et al. [15]. [1,4-^{14}C]-putrescine dihydrochloride (sp. act. 3.77 GBq/mmol) and *S*-adenosyl-L-[^{14}C-methyl]-methionine (sp. act. 2.08 GBq/mmol) were purchased from New England Nuclear, Boston, MA. Drugs administered to the patients for anaesthesia and during the operations were listed up in detail by RÖHER et al. [12a]. All solutions were prepared with twice distilled water which was further purified by a Milli-Q system (Millipore®, Bedford, MA).

Methods

Determination of histamine. The method of SHORE et al. [12] was modified for smaller tissue samples and the assessment of individual recovery rates for each tissue separately by using the internal standard procedure. 150–250 mg of the frozen material were suspended in 2 ml 1 *M*

Table 1
General characteristics of the patients in study, their diseases and operative treatment.

Tissue	Patients		Diseases	Operations
	Sex (m/f)	Age $\tilde{x}$ (range)		
Lung (5)[a]	5/0	57 (44–61)	Bronchial carcinoma (4), metastasis of hypernephroid tumour (1)*	Lung resection (5)
Heart[b] (4)	2/2	50 (44–60)	Mitral valve defect	Heart valve prothesis
Stomach[c] fundus (5)	5/0	61 (46–74)	Gastric carcinoma (4), gastric neurilenoma (1)	Total gastrectomy (4), local excision (1)
Stomach corpus (11)	9/2	59 (34–69)	Gastric carcinoma (7), peptic ulcer (3), parcreatic carcinoma (1)	Total gastrectomy (4), gastric resection (6), Whipple's operation (1)
Stomach antrum (9)	8/1	60 (46–62)	Gastric carcinoma (7), pancreatic carcinoma (2)	Total gastrectomy (4), gastric resection (3), Whipple's operation (2)
Duodenum (9)	6/1	50 (34–62)	Gastric carcinoma (2), ulcus ventriculi (1), pancreatic carcinoma (3), retroperitoneal metastasis of renal tumour (1)	Total gastrectomy (2), gastric resection (1), Whipple's operation (3), intestinal resection (1)
Jejunum (10)	6/3	60 (46–64)	Gastric carcinoma (6), pancreatic carcinoma (2), retroperitoneal metastasis of renal tumour (1)	Total gastrectomy with interposition of jejunum (4), gastric resection (1), Whipple's operation (3), intestinal resection (1)
Ileum (8)	3/5	58 (22–73)	Carcinoma of the right colon (2), Crohn's disease (5), small bowel tumour (1)	Right hemicolectomy (3), resection of the terminal ileum and coecum (4), intestinal resection (1)
Colon (8)	4/2	70 (24–73)	Colon carcinoma (2), Crohn's disease (3), diverticulitis (1)	Right hemicolectomy (3), resection of ileum and coecum (2), left hemicolectomy (1)
Sigma (9)	6/3	70 (49–75)	Sigma carcinoma (2), rectal carcinoma (5), diverticulitis (1), Crohn's disease (1)	Colon resection (5), left hemicolectomy (1), proctectomy (3)
Rectum (2)	2/0	54 + 59	Rectal carcinoma (2)	Low anterior resection (2)
Pancreas (2)	2/0	60 + 61	Pancreatic carcinoma (head) (2)	Whipple's operation (2)
Liver (11)	4/8	59 (34–73)	Gallstones (9), acute cholecystitis (1), gastric carcinoma (1)	Cholecystectomy (10), gastrectomy and liver excision (1)
Spleen (6)	3/3	32 (22–62)	Gastric carcinoma (2), Hodgkin's disease (3), intraoperative splenic rupture (1)	Gastrectomy and splenectomy (2), staging operation (3), splenectomy (1)

[a] Numbers of specimens, patients with the particular diseases and operations.
[b] Papillary muscle of the left ventricle.
[c] In the stomach and whole gut only mucosa specimens were taken.
m = male, f = female.

$HClO_4$, homogenized with an Ultraturrax homogenizer (microshaft TP 10 N) and centrifuged at 48,000 × *g* and 0–2°C for 30 min (Sorvall RC2-B). The supernatant was divided into a 0·8 ml aliquot for histamine assay and a 0·8 ml aliquot to which exogenous histamine in appropriate amount was added in a volume of only 0·1 ml 1 *M* $HClO_4$ for estimating the recovery rate. Both samples were filled up to 2.0 ml with 1 *M* $HClO_4$ and added to the usual mixture of n-butanol, NaOH and NaCl in the first step of the Shore procedure. However, long and tight Corning glass tubes (Sovirel®, 18 × 180 mm, 25 ml, with screw cups, Corning glass works, Wiesbaden) and a Heidolph shaking apparatus were used for the 3 extraction steps. By this equipment all centrifugations after the extractions were proved to be unnecessary which shortened the procedure considerably. The shaking time for sufficient extraction was 20 min in the first, 2 min in the second and 6 min in the third step of the method. Histamine ended in 4·5 ml 0·1 *M* HCl of which 2 ml were added to the usual mixture for the condensation reaction with *o*-phthaldialdehyde (OPD). Special emphasis should be focussed on the observation that the concentration of 0.1 ml 1% (w/v) OPD was absolutely necessary with such amount of tissue to provide a high, precise and reproducible recovery rate. Apparently reactants, other than histamine and biogenic amines, especially peptides bind the OPD to such an extent that in some cases only 40% recovery was observed with 0·1 or 0·2% OPD. The individual recovery rate for each tissue was recorded in a quality control chart in the exact time sequence of the assessment and analysed for systematic errors at the end of the study. Excluding these errors which very seldom occurred the mean recovery rate for each tissue was calculated and used for correcting the losses of histamine during the whole procedure. The range of recovery

rates for all tissues was 56–98% (!), but 84% on the average (cf. SHORE et al. [12]). The tissue histamine content was calculated with the aid of a calibration curve constructed from data obtained on 5 different days with authentic histamine in 0·1 *M* HCl by the following formula:

$$HC\ (\mu g/g) = (FU_S - FU_{BL}) \times 12 \times 4.5 \times \frac{10}{8} \times \frac{100}{REC} \times \frac{2}{0.8} \times \frac{1000}{SW}.$$

HC = histamine content, FU_S = fluorescence intensity (arbitrary units) of the sample, FU_{BL} = fluorescence of the blank, 12 = calibration factor, 4.5 = ml 0.1 *M* HCl at the end of extraction, 10/8 = aliquot of butanol phase before the last extraction step, REC = mean recovery rate, 2/0.8 = correction for dividing the supernatant after centrifugation, and SW = weight of the sample in mg tissue. Histamine was expressed in μg histamine dihydrochloride/g wet weight.

Measurement of DAO activity. The 1–2 g aliquot of the frozen material provided for determining DAO activity was homogenized usually with 19 volumes of ice cold 0·1 *M* sodium potassium phosphate buffer (pH 7.0) with an Ultraturrax homogenizer (microshaft TP 10 N). Only tissues with low enzymic activity such as lung, stomach and liver were used in a higher concentration (5 volumes of buffer) to detect any DAO activity. The suspension was centrifuged in a Sorvall RC2-B centrifuge at 48,000 × *g* and 0–2°C for 30 min and the supernatant was used as the source of the enzyme for the DAO assay according to KUSCHE et al. [13] who modified the test of OKUYAMA and KOBAYASHI [14] with ^{14}C-putrescine as substrate. As incubation times 10, 20, 40 and 60 min were chosen. The enzymic activity was calculated from those 3 consecutive reaction velocities which corresponded to linear reaction kinetics. To improve the specificity of the assay an additional blank was introduced by adding 2.5 μmol of the strong DAO inhibitor aminoguanidine to one of the test samples (incubation time 30 min). The enzymic activity was expressed in nmol/(min × g fresh weight) and not in nmol/(min × mg protein) to permit a comparison between the data in this communication and those previously reported. The transformation of the c.p.m./min-values into those corresponding to the definition of enzymic activity was permitted by calibration of the isotope assay with the coupled optic test (for references see KUSCHE et al. [7, 13]).

Measurement of HMT activity. Similarly to the assay of DAO activity a 1–2 g aliquot already prepared in the operation theatre (see previous description) was suspended in 4 ml 0·05 *M* sodium phosphate buffer (pH 7.4), homogenized and centrifuged as the samples used for determining DAO activity and assayed for HMT activity using the isotope test of BARTH et al. [15]. The enzymic activity was expressed in pmol/(min × mg protein), again in agreement with previous reports on the enzymic activity [15].

Protein determination. The biuret method adapted to the measurement of proteins in tissue samples (REINHOLD [16]) was used for testing HMT activity. In this modification a 'tartrate blank' was used in which only copper sulphate was not added to the reaction mixture.

Statistics. The median-range system and in a few exceptions also the mean-S.D.-range system were used for descriptive statistics.

Results

1. Problems in conducting the clinical-biochemical study and in adhering to the study protocol

The criteria for reliable sample-taking could be fulfilled often only with great difficulties. All specimens were taken and prepared by only the one surgeon in charge who either operated himself, assisted at the operations or was called to the operation theatre in time before the organ or tissue was removed from the patient. However, the time between withdrawal of the tissue block and freezing ranged from 1 to 20 min (Table 2) which was due to a number of difficulties in sample-taking and preparation. Only 1 min elapsed for excising a specimen from the liver, but preparing samples from the intestinal tract was by far more time-consuming. The tissue had to be examined macroscopically for defining the size of the tumour before the mucosa could be separated from healthy areas of the preparation. In addition the dissection of the material for purpose of the study should not interfere with the pathological examination. If the surgeon did not arrive in time, was unable to come for another operation or could not prepare the sample within 20 min then the specimen under study was discarded which happened in 20% of the cases primarily selected for the trial.

Table 2
Time of ischaemia during surgery and after removal of the preparation until freezing.

Tissue	Time during surgery[a] (estimated) $\tilde{x}$ min	Time after removal[b] (measured) min ($\tilde{x}$ (range))
Lung	30	10 (2–20)
Heart	0	1 (1/2–4)
Stomach – fundus	40	15 (5–15)
– corpus	30	5 (5–20)
– antrum	30	15 (5–20)
Duodenum	30	15 (5–20)
Jejunum	0	10 (5–20)
Ileum	20	10 (10–20)
Coecum	20	17.5 (10–20)
Colon	20	10 (5–20)
Sigma	20	10 (5–15)
Rectum	30	10 (10–10)
Pancreas	40	15 (15–5)
Liver	0	5 (3–15)
Spleen	15	10 (5–10)

[a] 'Warm ischaemia'. [b] 'Cold ischaemia'.
For definition of the two phases of ischaemia and methods for their assessment see text in Materials and methods.

A separate problem which was more difficult to handle was the time of 'warm' ischaemia as defined in methods. The time between ligation of supporting vessels and removal of the organ from its *in-situ* position depended on the type of operation, on the training and skill of the operating surgeon, difficulties in preparation due to adipositas of the subject or adhesions of tissues due to earlier operations or inflammating reactions and finally due to the spread of a tumour. Thus only for wedge-shaped excision of a piece of tissue from the liver or for jejunal mucosa which was taken during jejunum interposition after gastrectomy from areas with sufficient blood supply the time of warm ischaemia was negligible short. For gastric mucosa and pancreatic tissue, however, the warm ischaemia lasted for 40 min (Table 2). This problem in sample-taking was recorded, but could not be overcome in the course of the study. Conclusions on the accuracy of the data on histamine content and metabolism have therefore to be drawn with some caution.

2. Histamine content in human tissues (Table 3)

The histamine content in human tissues varied considerably from tissue to tissue and from individual to individual. On the average, it was high in the entire gastrointestinal tract, medium in lung, pancreas, colon and spleen and low in heart and liver. In general, the median and mean values corresponded well to each other indicating a normal distribution of the histamine content in tissues. This was already shown in a larger sample of biopsies obtained from the gastric corpus mucosa of 46 subjects [17]. In addition, the standard deviation did not exceed 40% of the mean in nearly all of the tissues investigated which is not in disagreement with a normal distribution of the values.

3. DAO activity in human tissues (Table 4)

The DAO activity in human tissues varied even more than the histamine content from tissue to tissue and from individual to individual. For instance, the range of the values obtained from human antrum and sigma mucosa covered a 20-fold interval and between several tissues the range of DAO activity stretched from O to several hundred mU/g.

For the first time very high activities of DAO were demonstrated in the *duodenal mucosa* unequivocally. These activities were the highest measured in human tissues under normal conditions. They declined from oral to aboral in the intestinal tract. The relatively high activities of the enzyme in the human corpus mucosa were also astonishing and probably due to an intestinalization (metaplasia) in stomachs with cancer or gastric ulcer. This finding may be of considerable importance in the decrease of acid secretion in these two diseases.

Very low activities of DAO or no activities were detected in several tissues. Deliberately no inhibitor of the aldehyde dehydrogenase was added to the incubation mixtures. This enzyme oxidizes the intermedium product of the ^{14}C-putrescine test (γ-aminobutyraldehyde) before cyclization prohibiting thus the extraction of radioactivity into the organic phase. However, only in the liver until now an interference of this

Table 3
Histamine content in human tissues.[a]

Tissue	n	$\tilde{x}$	Range	$\bar{x}$	S.D.
Lung	5	11.3	5.7–25.8	14.1	8.1
Heart	4	1.8	1.3–5.9	2.7	2.2
Stomach – fundus	5	34.6	16.3–38.2	30.0	9.0
– corpus	11	29.1	13.3–43.6	29.1	10.5
– antrum	9	27.9	13.6–43.0	26.5	10.2
Duodenum	8	23.1	13.1–41.0	23.7	8.1
Jejunum	10	29.8	17.4–42.4	28.0	8.4
Ileum	8	31.1	19.4–48.8	32.2	10.6
Colon	8	14.1	10.7–41.6	18.8	10.8
Sigma	9	31.7	16.2–41.3	29.7	10.6
Rectum	2	30.3	20.6–39.9	30.3	13.7
Pancreas	2	11.4	9.8–12.9	11.4	2.2
Liver	11	1.7	1.0–6.4	3.0	1.7
Spleen	6	6.3	3.4–10.9	6.4	2.6

[a] Values are expressed in μg/g wet weight.

Table 4
DAO activity in human tissues.[a]

Tissue	n	$\tilde{x}$	Range	$\bar{x}$	S.D.
Lung	6	0.0	0.0–0.4	0.1	0.0
Heart[b]	–	–	–	–	–
Stomach – fundus	5	0.6	0.0–2.3	0.8	1.0
– corpus	8	3.0	0.0–6.7	2.7	2.0
– antrum	9	12.0	3.0–65.0	18.6	20.6
Duodenum	9	133.0	36.0–226.0	127.8	52.5
Jejunum	10	131.5	36.0–190.0	118.4	55.3
Ileum	8	121.5	43.0–323.0	154.5	97.5
Colon	8	54.0	24.0–95.0	54.9	26.2
Sigma	9	67.0	5.5–103.0	58.2	31.6
Rectum	3	65.0	44.0–93.0	67.3	24.6
Pancreas	2	0.2	0.0–0.4	0.2	0.0
Liver	11	0.0	0.0–0.0	0.0	0.0
Spleen	6	0.9	0.4–2.0	1.0	0.6

[a] Activities are expressed in nmol/(min × g wet weight).
[b] Heart material was not sufficient to permit DAO assay.

enzyme with the DAO activity was shown [7]. The amount of heart tissue was so small to permit only histamine assays.

With very few exceptions again median and mean values were very similar in the different tissues. The most striking example for a rather skewed frequency distribution of the values was the human antrum mucosa. This finding is clinically relevant since differences between groups of healthy subjects and patients with peptic ulcer have to be carefully analyzed with appropriate statistical methods, not just by the usually applied Student's *t*-test.

4. HMT activity in human tissues (Table 5)

HMT activity was measured only in samples the amount of which was sufficient for determinating of all three parameters which were investigated in this communication. Thus only 70 organs remained out of 106 in which HMT was assayed, but no selection bias was detectable which could interfere with the interpretation of the data.

HMT activity varied much less than DAO activity from tissue to tissue and from individual to individual. There was no organ which did not contain any enzymic activity. In addition the range of the values obtained from different individuals was remarkably small in many tissues.

HMT activities were reported for most of the human organs for the first time, especially in the intestinal tract and among those tissues in the duodenum which is such an interesting organ in peptic ulcer pathophysiology. By far the highest activities of any tissue were found in the liver which is in agreement with BARTH et al. [1]. The lowest activities of the enzyme were determined in the stomach and in the lung, but rather high activities in the whole large bowel. Any function of the enzyme in these tissues is completely unknown.

The frequence distribution of the enzymic activities in a particular tissue was partly normally distributed, partly considerably skewed as shown by the shift of the mean from the median to the right and by a large standard deviation, e.g. in the ileum. This finding introduces caution in any interpretation of experiments on the pathological significance of HMT in man.

Table 5
HMT activity in human tissues[a]

Tissue	*n*	$\tilde{x}$	Range	$\bar{x}$	S.D.
Lung	5	44.1	13.7–57.3	38.9	17.9
Heart[b]	–	–	–	–	–
Stomach – fundus	3	25.8	25.8–48.6	33.4	13.2
– corpus	8	23.7	11.4–73.0	31.6	21.1
– antrum	7	32.4	10.8–49.9	28.1	14.2
Duodenum	5	59.9	44.7–93.3	68.1	20.0
Jejunum	8	37.9	18.8–72.1	42.5	20.7
Ileum	7	37.0	19.0–181.9	77.0	70.3
Colon	5	130.9	50.7–214.2	123.0	62.7
Sigma	9	161.9	41.8–244.4	161.5	65.5
Rectum	3	120.7	105.2–154.2	126.7	25.0
Pancreas[b]	–	–	–	–	–
Liver	5	669.1	281.7–1030.3	653.2	251.4
Spleen	5	102.8	34.5–117.7	83.4	39.2

[a] Values are expressed as pmol/(min × mg protein).
[b] No material was left for HMT assay. This was also the case in some other organs, thus explaining the discrepancies in number of various organs. For details see text in section 4 of Results.

Discussion

There is a long-lasting tradition in histamine research to measure alterations of histamine content and activities of histamine-forming and -inactivating enzymes in tissues as an index of histamine function in pathological states (for surveys see LINDELL and WESTLING [18], SCHAYER [19], BEAVEN [20], MASLINSKI [21], NEUGEBAUER and LORENZ [22]). In the last 30 years, however, the methodology of clinical trials has experienced such a substantial progress in preventing a series of biases [23] that most of the older work must be critically evaluated and repeated since very often prognostic factors were mixed in them in an unpredictable manner. Criteria for assessment of these studies include reliable methods for histamine assay and tests on enzymic activities in histamine metabolism, accurate and precise methods for sample-taking and -preparation and skilful and sufficiently complete collections of defined and useful attributes in patients and experimental animals [23]. Only when these criteria can be fulfilled a causative role of histamine may be suggested in a particular disease [24].

In a series of communications our group has concentrated its interest on the reliability of the methods for histamine and its metabolizing enzymes [11a, 13, 15, 17, 25–28]. Other workers in this field have pursued similar aims such as BEAVEN and his coworkers [20, 29] and HAKANSON et al. [30]. As a consequence, histamine levels, especially in body fluids, become lower and lower since the specificity of the assays could be improved. The criterion of accurate

sample-taking and preparation, however, was less often investigated [23, 27], but the problem is rather old [31]. Ischaemia leads to histamine release and inactivation and as a consequence of that to lower histamine values [32]. Therefore appropriate sample-taking *increases* histamine levels, and this may be confusing for the reader who is not aware of the two different principles which affect the data in opposite directions.

To exclude the problems of histamine assays as far as possible in studies on sample-handling the data of our own group using the same assays were compared mainly in this presentation (Table 6). However, for measuring histamine contents in tissues the bio-assay is equally suitable compared to the fluorometric assay [12, 26] which allowed us to include the data published by STONE et al. [33] and KUMAR et al. [34]. The data on DAO activity provided by BAYLIN et al. [8] and on HMT activity found by PEDEN et al. [4] cannot be compared with the present results because the tests for measuring the enzymic activities were different. The relative activities compared to a standard tissue, however, can be calculated by all these methods which may be helpful in studying at least the ranking of the various enzymic activities. For direct comparison of the enzymic activities, however, again earlier studies from our own group were used in which the problem of sample-handling was less satisfactorily solved than in the present communication (Table 6).

The histamine content in many organs assessed in this paper was at variance to earlier reports. This was especially true for the gastric mucosa where the differences amounted to about 100%. In the intestinal tract mucosal and full-wall thickness had to be compared which was difficult to accomplish, but the very low values of LORENZ et al. [26] have to be seriously doubted. In the liver, however, the earlier and present data of our group were in good agreement, but at variance by several hundred per cent to the results of KUMAR et al. [34] who used material from corpses. Also the histamine content of the spleen seemed to be considerably higher in the present article than in the earlier report [26] and in the study of STONE et al. [33] who used corpses like KUMAR et al. [34].

Even more astonishing than the histamine data were those obtained for DAO activity (Table 6). Much higher activities of the enzyme were found in the present study than in the earlier report (difference several hundred per cents!) as far as the intestinal tissues are concerned. In the stomach and spleen, however, the values of the two studies agreed very well. This may be explained by the suggestion that tissue hypoxia and ischaemia affected the enzymic activity in different organs to a different extent. This observation made the prognostic factor of tissue ischaemia for clinical trials rather unpredictable which therefore needs to be carefully followed-up and described in detail in the clinical studies [6].

In contrast to histamine content and DAO activity the HMT activity did not reveal great differences between the data of the present study and earlier data [22, 33a]. The lower values measured in the gastric mucosa in the present study than in the earlier report cannot be explained on the basis of different times of ischaemia. The results of BARTH [33a] are in agreement with his data on biopsies of the same tissues [9] for which the time of warm and cold ischaemia was only about 5–7 min [23]. Thus an explanation of the different findings other than biological variation cannot be given at the present moment.

A problem which could not be satisfactorily solved in the present study was the so-called 'warm ischaemia', that was the time from interrupting the blood flow until removing the organ preparations from the situs which lasted for stomach, ileum and colon about 20–30 min and for the pancreas up to 40 min. THON [28], for example, found a decrease of the histamine content in biopsy specimens of the gastric mucosa of 30% within 20 min provided the specimens were not cooled. Using autopsy material usually the warm ischaemia is rather long-lasting after death and may last for hours even if the body is cooled down. The arguments of KUMAR et al. [34] that the histamine content of the tissues may be significantly changed by anaesthesia and operation, was refuted by LORENZ [35] already in 1972.

The well-defined and very short time of warm and cold ischaemia which has to be considered in sample-taking and -preparation of biopsy material favours this procedure as most accurate and precise for getting *actual* values of histamine content and DAO and HMT activities in gastrointestinal tissues. From many other tissues, however, such as lung, liver, pancreas and heart, biopsies cannot be obtained by routine procedures. If only surgical specimens are available the samples should be taken under stan-

Table 6
Histamine content, DAO and HMT activity in human tissues: comparison with the literature.

Tissue	Histamine[a]								DAO[a]				HMT[a]			
	Present results		LORENZ [26]		STONE [33]		KUMAR [34]		Present results		KUSCHE [7]		Present results		BARTH [33a]	
	n	$\bar{x}$	*n*	$\bar{x}$	*n*	$\bar{x}$	*n*	$\bar{x}$	*n*	$\bar{x}$	*n*	$\bar{x}$	*n*	$\bar{x}$	*n*	$\bar{x}$
Lung	5	14.1	9	24	3	33	68	13.9	6	0.1	–	–	5	38.9	2	88
Heart	4	2.7	–	–	3	2	–	–	–	–	–	–	–	–	–	–
Stomach – fundus[b]	5	30.0	4	14			–	–	5	0.8	–	–	3	33.4	–	–
– corpus	11	29.1	19	17	3	14	–	–	8	2.7	3	4.4	8	31.6	7	70
– antrum	9	26.5	15	9			–	–	9	18.6	6	20.1	7	28.1	6	94
Duodenum	8	23.7	3	10	–	–			9	127.8	–	–	5	68.1	2	64
Jejunum	10	28.0	6	11	–	–			10	118.4	–	–	8	42.5	1	35
Ileum	8	32.2	8	16	–	–	68	18.9	8	154.5	–	–	7	77.0	3	79
Colon	8	18.8	5	9	–	–			8	54.9	10	8.9	5	123.0	–	–
Sigma	9	29.7	13	7	–	–			9	58.2	10	9.7	9	161.5	5	53
Rectum	2	30.3	11	7	–	–	–	–	3	67.3	11	16.3	3	126.7	2	114
Pancreas	2	11.4	–	–	3	5	–	–	2	0.2	–	–	–	–	–	–
Liver	11	3.0	33	4	3	2	68	0.7	11	0.0	–	–	5	653.2	15	447
Spleen	6	6.4	3	3	3	3	–	–	6	1.0	1	1.3	5	83.4	1	59

[a] Histamine content, DAO and HMT activity are expressed in μg/g, nmol/(min × g) and pmol/(min × g protein) respectively.
[b] In our study and in the study of KUSCHE and BARTH only mucosa was taken in the whole gastrointestinal tract, whereas LORENZ took mucosa in the stomach but the whole wall in the intestinal tract. STONE and KUMAR did not describe whether they used mucosa or the complete wall of the gastrointestinal tract. KUMAR did not describe at all from which part of the gut the samples were taken.

dardized and optimized conditions. Sample-taking from autopsies should be avoided.

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