THE EXTRACTABLE HISTAMINE CONTENT OF GASTRIC MUCOSA AND LUNG BEFORE AND AFTER PARENTERAL HISTAMINE ADMINISTRATION*

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Edkins in 1906[4] stated that aqueous extracts of the pyloric and cardiac mucosa from the stomach of dogs produced an increase in gastric secretion when injected subcutaneously, while extracts of the fundic mucosa failed to do so. As a result, a second or gastric phase of gastric secretion dependent upon the liberation of "Gastrin" from the pyloric mucosa was proposed by Edkins and Tweedy.[5] In 1920 Popielski[12] demonstrated an increase in gastric secretion after the injection of histamine and found that extracts of fundic and pyloric mucosa and various other tissues stimulated gastric secretion. In 1932 Sacks, Ivy, Burgess, and Vandolah[13] reported the isolation of crystalline histamine from hog's pyloric mucosa and showed that aqueous extracts of these tissues were inactivated by incubation with histaminase. From this work it was suggested that Edkins' "Gastrin" was probably histamine.

Gavin, McHenry, and Wilson[8] also studied the distribution of histamine in the dog stomach and reported their results in eight dogs. These authors used a method of extraction described by Best and McHenry,[1] and assayed the depressor effect of extracts on etherized cats. The average content of the fundic mucosa was stated to be 92 mg. per kg., while the pyloric mucosa contained 63 mg. per kg. These authors concluded that 80 per cent of the total histamine content of the gastric mucosa was contained in the fundic area.‡ Thus the work of Gavin, McHenry, and Wilson[8] did not support the hypothesis of Edkins. More recently, Emmelin and Kahlson[6] reported assays of gastric mucosa from three cats and four dogs, and found a difference in histamine activity with the greater activity present in the fundic mucosa. Uvnäs[15] in 1943 was able to isolate from the pylorus a gastric secretory excitant which was shown to be a protein substance and not histamine. Furthermore, this excitant

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‡It is apparent in Gavin's paper that "fundus" is used to indicate the hydrochloric and pepsin-producing parts of the stomach. More recent terminology divides this part of the stomach into corpus and fundus, with the fundus being that part of the stomach above the incisura cardiaca. We shall use the latter terminology.

was not found in the fundus of the stomach except in traces.

The part played by naturally occurring tissue histamine in gastric physiology remains vague. Emmelin[6] suggests that histamine acts as a final link in the humoral mechanism, causing excitation of the parietal cells.

Our experiments were carried out to determine what changes might occur in the extractable histamine concentration of the gastric mucosa after the intramuscular administration of histamine in beeswax, as previously described by Code and Varco.[3] The extractable histamine in the lungs of the animals used was also determined and correlated with the above.

Methods

The thirteen animals used were mongrel dogs, young adults and adults of unestablished ages, debarked, and on a standard diet of "Purina Dog Chow" and water ad libitum. The dogs received one feeding per day in the early afternoon. Since each animal served as its own control, specimens of mucosa from the pylorus* and corpus and from lung were obtained for biopsy assay purposes at a preliminary operation. Under intravenous Nembutal anesthesia and with artificial ventilation of the lungs through an intratracheal tube, the right chest was opened by an intercostal incision in the sixth interspace. Re-section of the right lower lobe was carried out with individual silk ligations of the artery, vein, and bronchus, in the order named. Following repleuralization of the bronchial stump, the chest was closed and pneumothorax was reduced by aspiration. Immediately following closure of the chest, laparotomy was performed and biopsies of the pylorus and corpus of the stomach were taken. The defects in the stomach were closed transversely by inversion with catgut sutures in the mucosa and with interrupted silk sutures in the serosa. There was one operative death among the thirteen animals in the series. The tissues obtained were then subjected to immediate histamine extraction.

After a recovery period of from two to three weeks the dogs were given daily intramuscular injections of 30 mg. of histamine† in beeswax prepared after a method described by Code and Varco.[3] Injections were given in the leg muscle at 9:00 A.M. each day. The total number of daily injections varied from two to seventy-one (Table 1).

Upon death or sacrifice of the animals, post-mortem examination was performed with special reference to the lung, esophagus, stomach, and bowel. The left lower lobe and specimens of gastric mucosa from the areas adjoining the previous operative defect were removed and subjected to extraction. All specimens were obtained immediately upon sacrifice or in the event of spontaneous death as soon as possible, the longest interval being approximately ten hours. The time lapse between the last injection of histamine and removal of tissue was variable with a range of two to fifteen hours. The position of the control biopsy

*Pylorus is meant to include the pyloric antrum.

was always verified to insure that the area in question (pylorus and corpus) had been biopsied. Pyloric obstruction as a result of operation was looked for and ruled out in all animals. The bronchial stumps were inspected for leaks and ulceration. Microscopic sections of lesions were taken whenever indicated.

Extraction technique: The gastric specimens were prepared by dissecting mucosa from the muscularis. A one- to three-gram block of lung tissue was removed from the periphery of the excised lung lobe. After blotting dry the specimens were weighed to the nearest hundredth of a gram. The histamine extraction then proceeded according to a method described by Gilman and Lindskog.[9] The tissue, being first ground with white sand until fragmentation seemed complete, was transferred to a wide-mouthed bottle; 50 ml. of 70 per cent alcohol containing enough hydrochloric acid to make 0.1 normal solution were added, and the mixture was subsequently transferred to a 50 cc. tube and centrifuged for five minutes at high speed. The supernatant solution was poured into an evaporating dish and centrifugation repeated after adding 40 cc. of the alcohol to the sediment and stirring well. The solutions were evaporated to dryness on a steam bath and the residues were set aside until time for assay.

Assay technique: The dried residues were assayed for histamine activity according to a method described by Guggenheim and Löffler[10] using atropinized guinea-pig ileum suspended in 3 cc. of Tyrode's solution at constant temperature. The contractions produced by the dried residues diluted by volume in Tyrode's solution were then compared with contractions produced by a known histamine solution. The usual measurement and recording of contractions on a smoked drum were replaced by direct readings from a millimeter scale fixed in the arc of the writing lever point. Optimum range and sensitivity were estimated by establishing an action-concentration curve for each strip of gut as described by Emmelin, Kahlson, and Wicksell.[7] The quantities of unknown extract and standard solution used for comparison were kept at equal volume. The results are expressed as micrograms of histamine di-phosphate per gram of tissue.

Results

Five animals died spontaneously. The causes of death were acute perforated duodenal ulcer with peritonitis, acute duodenal ulcer with gastro-intestinal hemorrhage, acute perforated esophageal ulcer with empyema, and organizing duodenal ulceration with gastro-intestinal hemorrhage. Three animals were sacrificed and found to have acute hemorrhagic antral gastritis, organizing duodenal ulceration, and chronic duodenal and esophageal ulceration, respectively. One animal sacrificed after 71 daily injections of histamine-beeswax preparation was free of pathology at post mortem (Table 1).

The histamine content of the mucosa from the corpus, pylorus, and lung for each animal before and after injections of the histamine-

†Histamine donated by Pfanstiel Chemical Company and Hoffman-La Roche Incorporated.

TABLE 1

Dog	Wt.	Assay before histamine injection: pylor.	corp.	lung	No. Inj.	Assay after histamine injection: pylor.	corp.	lung	Death	Pathology
1.	12K	244*	240	173	14	240	240	180	spon.	acute perf. duodenal ulcer.
2.	13K	108	181	52	7	96	200	62	spon.	acute duo. ulcer and hemorrhage.
3.	10K	174	192	153	2	174	206	174	spon.	acute perf. esoph. ulcer and hemorrhage.
4.	10K				10	152	160	168	sacrif.	acute hemorr. antral gastritis.
5.	11K	102	100	108	32	112	116	122	sacrif.	organizing duodenal ulceration.
6.	18K	75	76	77	18	78	84	83	spon.	organizing duodenal ulcer and hemorrhage.
7.	8K	70	72	59	63	74	72	64	sacrif.	chronic duodenal and esophageal ulcers.
8.	8K	53	53	55	48	54	54	50	spon.	chron. perf. esoph. ulcer, empyema, chron. gastritis.
9.	14K	49	40	28	71	42	36	28	sacrif.	normal gastro-intestinal tract.
10.	13K	220	227	210	none	216	232	232	sacrif.	operative control; normal gastro-intestinal tract.
11.	18K	28	30	19	control					
12.	11K	40	40	40	control					
13.	8K	140	151	125	control					

*All figures are expressed in micrograms (histamine di-phosphate) per gram of tissue (wet weight).

beeswax preparation are compared in Table 1. It will be seen that the content of extractable histamine in the mucosa of the pylorus and that of the corpus are very similar, while the content of the lung tends to be in the same range for the individual animals. The differences in histamine level before and after histamine injection are within the range of experimental error for the method. The histamine concentration for individual animals in this group varied from 27.5 gamma per gram to 240 gamma per gram for the pyloric mucosa with the other tissues having a similar degree of variation.

Discussion

The results indicate that there is little change in the level of extractable histamine from gastric mucosa or lung parenchyma after prolonged injection of histamine-beeswax. It is further demonstrated that varying degrees of acute and chronic gastritis and peptic ulcer pathology do not significantly alter these findings. The histamine content in the mucosa of corpus and pylorus did not differ appreciably in the individual animal. These findings do not support the original work of Gavin, McHenry, and Wilson.[8] The results presented confirm the results of human post-mortem gastric studies previously reported from this laboratory by Bloomer.[2] His study of 19 cases showed that the fundic mucosa contained an average of 10.5 gamma per gram as compared with an average of 12.6 for pyloric mucosa. Trach, Code, and Wangensteen[14] also failed to demonstrate a consistent difference between fundic and antral mucosal histamine levels in man.

The absence of change in extractable histamine content, despite various and sometimes severe pathological changes (perforated ulcer, peritonitis, gastritis, and varying degrees of ulceration), correlates with the failure to demonstrate any appreciable change in histamine content in the lungs of dogs after prolonged daily injections of histamine in aqueous solution. In these animals gastric pathology was not observed.[11] The failure of gastric and lung histamine levels to be altered by prolonged injection of aqueous histamine and histamine-in-beeswax opposes the hypothesis that lung or stomach parenchyma has the ability to filter and selectively store circulating histamine.

The dog is known to secrete a more highly acid gastric juice than does man. The question whether or not this may be correlated with the higher histamine content of dog gastric mucosa has been raised. In the series of human cases studied by Trach, Code, and Wangensteen[14] there

was no correlation between the concentration of acid in the juice from the intact stomach and the histamine content of the gastric mucosa. It appeared, however, that in our series those dogs with the greatest histamine content developed the most acute and lethal pathology. This cannot be taken as conclusive, however, unless a larger series could be presented. A study has been performed by Trach, Code, and Wangensteen[14] in five patients with peptic ulcer of stomach or duodenum. The histamine content of gastric mucosa studied by these authors was not increased above the accepted normal range for gastric mucosa, as found by Bloomer in this laboratory at post-mortem examinations.

Summary

1. The extractable histamine content of the mucosa from the pylorus and corpus of the dog stomach is approximately the same and does not vary appreciably following intramuscular injection of histamine in beeswax over long periods of time.

2. The histamine content of the dog lung is similar in range for a given subject to that of the gastric mucosa and is likewise not altered significantly by injection of histamine in beeswax.

3. Injected histamine does not appear to augment the bound histamine of gastric mucosa, and therefore probably acts directly upon the secreting cells.

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