

# THE EFFECT OF CORTISONE ON GASTRIC HISTAMINE CONTENT AND GASTRIC SECRETION IN PYLORUS LIGATED GUINEA-PIGS

BY

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*(Received November 23, 1966)*

The tendency of prolonged corticosteroid therapy to cause peptic ulceration in man is well established, but the mechanism by which this occurs is still debated. Increased gastric acid secretion is considered essential in the pathogenesis of duodenal ulceration and it is generally agreed that if administered in large doses for considerable periods of time, corticosteroids may increase gastric secretion in susceptible individuals (Carbone, Liebovitz & Forsheim, 1957; Plainos & Philippu, 1958; Gray, 1961).

There are divergent views in the literature regarding the effect of corticosteroids on gastric secretion in animals. Some investigators (Zubiran, Kark & Dragstedt, 1952; McGee, Blackburn, Lance & Scott, 1958) have observed an increase in gastric acid secretion in dogs with denervated pouches following the administration of pharmacological doses of cortisone for prolonged periods of time; others (Dragstedt, Ragins, Dragstedt II & Evans, 1956; Wiederanders, Classen, Gobbel & Doyle, 1960) failed to observe any change in secretory activity. In acute experiments, Shay (1959) demonstrated that 100 mg hydrocortisone caused an increase in total acid output of dogs, while Ragins, Dragstedt II, Landon, Lyon & Dragstedt (1956) did not observe any effect following the administration of 200 mg cortisone in the same species.

It has previously been reported (Kovacs, 1965) and confirmed (Hicks, 1965) that prolonged administration of cortisone to guinea-pigs caused a significant fall in the histamine content of several organs. Histamine is known to be one of the most potent stimulators of gastric secretion and, according to Code (1956, 1965), it is the final, common local chemostimulator of gastric acid secretion. It was thought that cortisone administration may cause a release of histamine in the guinea-pig stomach, which in turn might lead to gastric hypersecretion.

The results presented here show that in the pylorus ligated guinea-pigs cortisone decreases gastric histamine levels and increases gastric secretory activity. Furthermore, a definite correlation between the duration of cortisone treatment and the intensity of the secretory response was observed.

## METHODS

### *Animals*

Male guinea-pigs weighing 320-340 g at the start of the experiments were used. They were of the multi-coloured short-haired variety, obtained from the Quebec Breeders Association (Canada).

The guinea-pigs were fed with a diet of Purina Guinea-Pig Chow, water and hay *ad libitum*. Before experiments they were fasted in cages with wide mesh wire bottoms; water was allowed at all times.

#### *Shay method*

Guinea-pigs were fasted for 28 hr and the pylorus was ligated under light ether anaesthesia. The animals were then returned to their cages and deprived of food and water till they were killed 18 hr later with an overdose of ether. Before respiration stopped, stomachs were ligated at the oesophageal end, removed and blotted dry on filter paper. A small incision was made in the greater curvature of each stomach and the gastric content emptied into calibrated centrifuge tubes. The stomach was then slit along its greater curvature, examined macroscopically for lesions and extracted for histamine 2 to 3 hr later.

#### *Analysis of the gastric juice*

The gastric content from each animal was centrifuged and volumes of solid matter and juice were noted. Those samples that contained 2 ml. or more of solid particles were discarded. The pH was determined by a direct reading pH meter. Free and total acidity were determined by titrating the samples with 0.1 N NaOH using Töpfer's reagent and phenolphthalein as indicators. Pepsin determinations were performed by the Anson haemoglobin method (Anson, 1938) as modified by Bucher, Grossman & Ivy (1945). The results are expressed as Peptic Units (PU).

#### *Estimation of histamine*

Intact animals were fasted for 18 hr before being killed by a blow on the head; they were bled from the jugular vein and their stomachs immediately removed. Pylorus ligated animals were prepared and killed as described previously. Each stomach was trimmed free of adipose tissue and rinsed under cold distilled tap water. It was blotted dry on filter paper and weighed as a whole, or divided into two sections. One section consisted of the antrum and pylorus (referred to as antrum), and the other section of the forestomach, cardiac region and corpus (referred to as corpus). Histamine concentrations were measured in duplicate by the fluorometric assay of Shore, Burkhalter & Cohn (1959), utilizing an Aminco-Bowman Spectrophotofluorometer with an excitation wavelength of 350 m $\mu$  resulting in fluorescence at 450 m $\mu$ . Histamine values refer to the base. Recovery of known amounts of histamine added to tissue homogenates was between 85 and 90%.

#### *Treatment*

Cortisone acetate (Cortone; Merck, Sharp & Dohme) in a suspension of 50 mg/ml. was used. Details of dosage and times of administration are given in Results. Pylorus ligated control guinea-pigs received corresponding volumes of saline subcutaneously.

#### *Statistical analysis*

In the statistical evaluation of the results, Student's *t* was used as a significance test.

## RESULTS

#### *Effect of cortisone on gastric histamine content in intact guinea-pigs*

Sixteen guinea-pigs were injected with 100 mg/kg cortisone acetate. Injections were made subcutaneously daily, in the morning, for 10 days. Twenty-four hours after the last dose, the animals were killed and histamine contents of the whole stomach, or antrum and corpus, were determined. The results, summarized in Table 1, show that the average histamine level recorded for the whole stomach of cortisone-pretreated animals was significantly lower than values obtained from control guinea-pigs. Average histamine contents of the antrum and corpus, which were the same in untreated guinea-pigs, were equally and significantly lowered by cortisone pretreatment.

TABLE 1  
GASTRIC HISTAMINE CONTENT OF GUINEA-PIGS FOLLOWING TREATMENT WITH CORTISONE

Animals were injected subcutaneously with 100 mg/kg cortisone acetate once daily for periods indicated in the Table. Histamine concentrations are expressed in terms of the base. Values are means with standard errors

Treatment	Animals (No.)	Histamine content ( $\mu\text{g/g}$ )		
		Whole stomach	Corpus	Antrum
None	15	10.20 $\pm$ 0.30		
Cortisone, 10 days	11	6.64 $\pm$ 0.22†		
None	5		9.92 $\pm$ 0.69	9.78 $\pm$ 0.80
Cortisone, 10 days	5		6.64 $\pm$ 0.34†	6.61 $\pm$ 0.66*

\*  $P < 0.01$ . †  $P < 0.001$ .

*Effect of cortisone treatment on gastric histamine content and gastric secretion in pylorus ligated guinea-pigs*

Eight guinea-pigs received a single injection of cortisone acetate (100 mg/kg subcutaneously) immediately after pylorus ligation. Six guinea-pigs were treated for three days and 12 guinea-pigs for 10 days with the same dose, given daily by subcutaneous injection. The last doses were administered immediately following pylorus ligation. Data obtained in these studies are presented in Table 2. As can be seen, a single injection of cortisone lowered the average gastric histamine content by 16.8%, three injections by 29.9% and 10 injections by 27.6% compared with values obtained from control pylorus ligated guinea-pigs.

Also summarized in Table 2 are results showing the effect of cortisone on volume, pH, free and total acidity of the gastric juice in pylorus ligated guinea-pigs. A single dose of cortisone did not alter the volume of the gastric juice secreted. The values obtained for the pH were lower, free and total acid output higher, but not significantly, when compared with control values. Following a three-day cortisone treatment, a small increase occurred in the volume of gastric juice secreted. The values for the pH have fallen significantly compared to control readings while free and total acid outputs were significantly higher. Ten pretreatments with cortisone produced a highly significant decrease in the volume and pH of the gastric juice and a highly significant increase in free and total acid output compared to control values. In animals treated for 10 days with cortisone the volume was also significantly lower, free and total acid concentration significantly higher when compared with results obtained following a three-day treatment with cortisone. Peptic activity was slightly higher than the corresponding average value recorded from guinea-pigs with pylorus ligation alone.

#### DISCUSSION

The technique of pylorus ligation described by Shay, Komarov, Fels, Meranze, Gruenstein & Siplet (1945) as a method of producing gastric ulceration in the rat was employed in the present work to study the effect of cortisone on gastric secretion in guinea-pigs. In rats, severe gastric ulceration is produced if the pylorus is ligated for 18 hr. In contrast, pylorus ligation over the same period of time in guinea-pigs did not lead to ulcer formation. Data in the literature on effects of cortisone on gastric histamine content and gastric secretion in rats also differ from present findings. Following glucocorticoid administration, Telford & West (1960) reported elevated histamine

TABLE 2

EFFECT OF CORTISONE ON GASTRIC HISTAMINE CONTENT AND GASTRIC SECRETION IN PYLORUS LIGATED GUINEA-PIGS

Guinea-pigs were injected subcutaneously with 100 mg/kg cortisone acetate once daily, for periods indicated in the Table. The last injections were administered immediately after pylorus ligation and all animals were killed 18 hr later. Determinations were made individually; figures represent mean values and standard errors. Histamine concentrations are expressed in terms of the base. In the second column figures in parentheses represent the number of stomachs assayed for histamine content. In the third column figures in parentheses represent percentage decrease in histamine content compared to control values. N.S. = Not significant

Treatment	Animals (No.)	Gastric histamine content ( $\mu$ g/g)	Volume of gastric juice	pH	Free HCl			Total acid		Peptic activity (PU/l.)
					m-equiv	m-equiv/l.	m-equiv	m-equiv	m-equiv/l.	
(a) Pylorus ligation + saline	15 (8)	8.29 $\pm$ 1.28	29.30 $\pm$ 1.72	4.08 $\pm$ 0.45	0.14 $\pm$ 0.06	5.07 $\pm$ 2.04	0.49 $\pm$ 0.07	17.31 $\pm$ 2.39	12.21 $\pm$ 0.62	
(b) Pylorus ligation + cortisone, 1 day	8 (8)	6.89 $\pm$ 1.05 (16.8)	31.21 $\pm$ 2.93	2.98 $\pm$ 0.67	0.38 $\pm$ 0.14	13.65 $\pm$ 4.82	0.82 $\pm$ 0.17	28.77 $\pm$ 6.13		
(c) Pylorus ligation + cortisone, 3 days	6 (5)	5.82 $\pm$ 0.20 (29.9)	34.08 $\pm$ 3.66	1.86 $\pm$ 0.08	0.65 $\pm$ 0.09	20.57 $\pm$ 3.93	1.17 $\pm$ 0.08	36.13 $\pm$ 4.10		
(d) Pylorus ligation + cortisone, 10 days	12 (5)	6.00 $\pm$ 0.36 (27.6)	20.97 $\pm$ 1.60	1.78 $\pm$ 0.13	0.80 $\pm$ 0.10	37.97 $\pm$ 3.54	1.21 $\pm$ 0.11	58.00 $\pm$ 3.60	13.79 $\pm$ 1.36	
P: (c) compared with (a)		N.S.	N.S.	0.001	0.01	0.01	0.001	0.001	0.001	
P: (d) compared with (a)		N.S.	0.01	0.001	0.001	0.001	0.001	0.001	0.001	N.S.
P: (d) compared with (c)		N.S.	0.01	N.S.	0.05	0.001	N.S.	0.001	0.001	

levels and Schayer (1956) found an increase in histamine binding in the rat stomach. Welbourn & Code (1953) have shown that in the pylorus ligated rat repeated cortisone injections caused significant decrease in gastric secretion; the same was reported by Haikonen & Räsänen (1965) using dexamethasone. However, the rat is an odd beast (Code, 1965), differing in many aspects from other experimental animals. The rat is, for example, the only species in which specific histidine decarboxylase (Hakanson & Owman, 1966) and diamine oxidase (Code, 1965) occur in the stomach. In the guinea-pig, cat, dog, man, but not in the rat, methylation of histamine in the ring is the principal pathway of histamine destruction in the stomach (Liepins, Ivy & Suzuki, 1958). In the rat histamine is not methylated but undergoes deamination in the gastric mucosa (Code, 1965). The rat is a corticosterone secretor, while in man, cat, dog or guinea-pig hydrocortisone is the principal adrenal hormone. These and similar differences between the rat and guinea-pig may account for, or contribute to, different responses observed in the two species regarding the effects of cortisone on gastric histamine content and gastric secretion.

The pattern of histamine release seems to be different in the guinea-pig stomach from that seen in other guinea-pig tissues. For example, the guinea-pig lung does not release histamine following a single dose of cortisone (Hicks, 1965) but a significant decrease can be achieved in pulmonary histamine content, if cortisone administration is maintained for at least 10 days (Kovacs, 1965). In contrast, administration of a single dose of cortisone already led to a fall in the gastric histamine content of pylorus ligated guinea-pigs. The lowest histamine levels were obtained after three days of treatment, and histamine levels were not lowered further by continued cortisone administration. It appears (Kahlson, Rosengren & Thunberg, 1963) that in tissues rich in mast cells, a histamine molecule once formed is firmly held and has a long intracellular lifetime, whereas in non-mast-cell-tissue, such as the gastric mucosa, the reverse is true, and the actual histamine content represents the balance between the rate of formation and rate of removal of histamine.

The results described in this paper not only confirm data reporting that cortisone may increase gastric acid secretion, but they also show a concomitant decrease in gastric histamine content and a correlation between the duration of treatment and intensity of gastric hypersecretion. As the number of cortisone treatments were increased, acid output increased progressively; a net loss of histamine, however, was not observed beyond that seen following three cortisone administrations. Thus, on the basis of present findings, no direct causal relationship could be established between cortisone treatment, histamine release and gastric hypersecretion. It remains to be seen if further studies involving gastric histamine turnover rates and the use of inhibitors of histamine formation and destruction can lead to the establishment of such a causal relationship.

#### SUMMARY

1. The effect of cortisone on gastric histamine content and gastric secretion was studied in guinea-pigs. Cortisone acetate was injected subcutaneously in one daily dose of 100 mg/kg for 1, 3 or 10 days.

2. Cortisone injected for 10 days produced a significant fall in histamine levels of the intact stomach.

3. In pylorus ligated guinea-pigs, administration of a single dose of cortisone already produced a fall in the pH and a slight increase in free and total acidity of the gastric juice. Three cortisone injections were found to alter these parameters significantly when compared with values obtained from control pylorus ligated guinea-pigs. Ten cortisone pretreatments further reduced the pH and increased free and total acid output, all changes being highly significant.

We are indebted to the Medical Research Council of Canada for the support of this work by a grant to Eva Kovacs. We also wish to thank Dr. W. Dorian of Merck, Sharp & Dohme for generous supplies of cortisone injections.

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