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Histamine gastric ulceration in the guinea-pig. Some observations on a new method

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Consistent gastric ulceration can be produced in suitable strains of guinea-pig, after high duodenal ligation under pentobarbitone anaesthesia, by the subcutaneous injection of a relatively low dose of aqueous histamine acid phosphate. The ulceration coincides with a dose of histamine which produces sub-maximal secretion volume and which is greater than that producing the maximum secretion. The method does not require antihistamine cover, and it is shown that antihistamines may complicate the true histamine response by the stomach. The results provide evidence that this type of ulceration follows the action of gastric juice on a functionally impaired mucosa.

SINCE Code and his colleagues (Code & Varco, 1940, Hay, Varco, Code & Wangensteen, 1942) first studied the use of a long-acting non-aqueous intramuscular injection of a suspension of histamine dihydrochloride as an ulcerogenic agent, several workers (Crane, 1947; Ambrus, 1951, 1953; Harrison, 1955) using oily suspensions of histamine, have used the guinea-pig in this type of experiment for studies in peptic ulceration. The animal is sensitive to histamine which induces a copious gastric secretion readily obtainable by stomach tube without surgery (Watt, 1955); and it is possible to produce ulceration without causing death.

Halpern & Martin (1946) and Zaidi & Mukerji (1958) on the other hand, used a relatively large dose of the faster-acting aqueous solution of histamine hydrochloride given intraperitoneally, at the same time protecting the animals with the antihistamine promethazine given by the same route.

Both methods have limitations: they normally require the use of high doses of histamine which necessitate protection of the animals either by delaying the release rate by using a wax-oil base or by concurrent use of antihistamine drugs or both. Antihistamines may modify the gastric effects of histamine to some extent, although it has been widely assumed that the ulcerative and secretory effects of histamine on the stomach are, in general, unimpaired, the drugs merely eliminating the fatal systemic effects of histamine otherwise liable to occur with such dosage.

The Shay method (Shay, Komarov, Fels, Meranze, Gruenstein & Siplet, 1945), which uses the rat, is based on the fact that gastric juice retained in the stomach as a result of pyloric ligation is a principal aetiological factor in this type of experimental gastric ulceration. These authors stated that gastric ulcers may also be produced in the guinea-pig by the method described for the rat. However, experimental details for the guinea-pig were not given and furthermore, the method for the rat required about 18 hr of unanaesthetized ligation. In the method to be described we have used the guinea-pig for studying experimental peptic ulceration. This is produced by high duodenal ligation with subcutaneous injection of aqueous histamine acid phosphate in a dose small enough of

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itself to permit indefinite survival of the animal without resort to antihistamines, which, although frequently used in this type of study, complicate the picture of histamine action on the stomach of the guinea-pig.

Experimental and results

Male albino guinea-pigs, 250–700 g body weight, were fasted for 18–24 hr in conditions preventing coprophagy; water was allowed *ad lib.* They were anaesthetised by pentobarbitone sodium, 30 mg/kg intraperitoneally. A single mid-line abdominal incision (2–3 cm) was made, carefully avoiding damaging the stomach when entering the peritoneal cavity. The pyloric end of the stomach was located and the duodenum exteriorised without disturbing the stomach. An artery clip was placed on the duodenum above the opening of the bile duct avoiding interruption of blood supply. This constriction ensures retention of the whole gastric secretion; it also prevents regurgitation of duodenal contents which might interfere with the effect of the acid secretion. The peritoneal cavity was closed around the protruding artery clip.

Aqueous solution of histamine acid phosphate (2·5–7·5 mg/kg depending on the strain used) was injected subcutaneously, the control animals receiving the same volume of normal saline after undergoing the same operative procedure. The animals were left undisturbed in a supine position on a warm table and 1 hr after the injection of histamine they were killed with ether. The stomach was carefully removed with the clip in position, washed, and the gastric secretion collected, centrifuged, its volume measured and free and total acidities titrated with Topfer's reagent and phenolphthalein respectively. Volume of juice was reported as ml/kg body weight. The stomach was then distended with tap water injected through the cardiac orifice and examined against a good light for ulceration. The severity of ulceration may be graded according to the following scale: +, a few small ulcers (up to four); 2+, several small ulcers (five to eight); 3+, many small ulcers (9–16) and a few large ulcers; 4+, large areas of ulceration with confluence or more than 16 small ulcers, or impending perforation. Average group scores were calculated.

Ulcers were observed singly, in groups, or as diffuse ulcerated regions. Although ulcers are found in the antral region and less frequently on the lesser curvature, they usually appear in the fundic region and along the line of greater curvature indicating that the glandular portion of the stomach, which possesses the highest secretory activity, appears to be most susceptible. Two types of ulcers were observed; diffuse ulcerated regions and small round punched out areas, both types occurring in all ulcer-bearing regions. The latter type had a clean transparent floor surrounded by a whitish lining of mucus. Haemorrhage was not a common feature and only occurred when ulceration was severe. Non-ulcerated regions occasionally showed a superficially eroded mucosa but in most instances, these areas and tissues peripheral to an ulcer were apparently healthy.

The dose of histamine acid phosphate (which varied from 2·5–7·5 mg/kg) giving a consistent average ulcer picture of 3+ to 4+ was ascertained for

each strain after the animals had been maintained exclusively on diet 18 and water for at least one week immediately before the experiment. The diet was therefore relatively low in ascorbic acid; nevertheless, the animals appeared healthy and showed no signs of scurvy.

Control animals did not show ulceration, although secretions were acid. Differences in volume and in free and total acidity between control and test groups were significant (Table 1).

TABLE 1. HISTAMINE GASTRIC ULCERATION AND SECRETION IN DIFFERENT STRAINS OF GUINEA-PIG

Strain	No. of animals	Dose of histamine acid phosphate mg/kg s.c.	Average ulceration	Average volume of secretion (ml/kg)	Average acidity of secretion (m-equiv./litre)	
					Free	Total
P	5	2·5	1·4+	40	114	120
T	6	2·5	1·6+	35	118	131
P	12	5·0	3·2+	30	98	105
T	6	5·0	1·7+	25	100	108
P	5	Control	0	15	54	63
T	7	Control	0	17	59	67

T = resistant strain; P = susceptible strain. Significance of differences between controls and histamine-treated: volumes $P<0{\cdot}02$; acidities $P = 0{\cdot}001$–$0{\cdot}05$.

EFFECT OF ANAESTHETIC

Pentobarbitone sodium, 30 mg/kg intraperitoneally, was chosen in preference to urethane because when urethane, 1·25 g/kg intraperitoneally, was used with histamine, 2·5 mg/kg subcutaneously, ulceration appeared to be much more severe. The 10 animals given pentobarbitone had average ulceration of 0·2+; average secretion 35 ml/kg and average acidity of secretion (m-equiv./litre) free 109, total 117. Corresponding figures for the 8 animals given urethane were 2·4+; 30; 114 and 121. An ulcer-resistant strain which consistently failed to show ulceration with 2·5 mg/kg histamine acid phosphate when pentobarbitone was used, did so with urethane, with the same dose of histamine (and even in the absence of histamine in certain preliminary control experiments).

STRAIN DIFFERENCE

Differences in response by different strains of guinea-pig were observed. In the more susceptible (P) strain, 2·5 mg/kg histamine acid phosphate increased the volume and free and total acidity of gastric secretions, and produced low degree of ulceration, while at 5·0 mg/kg significant increase in ulceration associated with a slight decrease in volume and acidity was observed (Table 1). The resistant (T) strain behaved similarly except that the high dose did not increase ulceration. Table 1 further shows that for both resistant and susceptible strains, the volume of gastric juice and its acidity are not maximal when the dose of histamine is high enough to cause ulceration.

ROLE OF FREE ACID IN STOMACH CONTENTS

In the same type of experiment using the susceptible (P) strain, aluminium hydroxide gel B.P. (2 ml) was introduced into the stomach by

tube after ligation of the duodenum and subcutaneous injection of histamine acid phosphate 5 mg/kg. Control animals received water (2 ml). Acidity of gastric secretion was eliminated, the 11 animals receiving gel having average ulceration of 0·4+; average volume of secretion 27 ml/kg; average acidity of secretion (m-equiv./litre) free 0, total 0. Corresponding figures for the 5 controls were 4+; 31; 92 and 100.

EFFECT OF ANTIHISTAMINES

In attempting to intensify ulceration in the resistant (T) strain, a higher dose of histamine (7·5 mg/kg) was employed with the antihistamine promethazine hydrochloride, 1 mg/kg intramuscularly, 30 min before. The promethazine gave this strain complete protection against gastric ulceration. The susceptible strain, receiving 5 mg/kg histamine acid phosphate, was also protected by the drug ($P<0·001$). Antihistamine protection by promethazine is apparently not associated with significant change in total volume or acidity (Table 2). Similarly, the specific anti-

TABLE 2. EFFECT OF ANTIHISTAMINES ON HISTAMINE ULCERATION AND SECRETION IN AN ULCER-RESISTANT AND AN ULCER-SUSCEPTIBLE STRAIN OF GUINEA-PIG

Strain	No. of animals	Dose of histamine acid phosphate (mg/kg s.c.)	Average ulceration		Average volume of secretion (ml/kg)	Average acidity of secretion (m-equiv./litre)	
			With promethazine	Without 1 mg/kg i.m.		Free	Total
T	6	7·5	0	–	33	119	124
P	15	5	+	–	30	108	116
			Mepyramine maleate 5 mg/kg i.m.				
T	7	7·5	+	–	47	116	125
P	16	5	1·6+	–	41	119	126
T	6	7·5	–	3·3+	30	87	97
P	12	5	–	3·2+	30	98	105

T = resistant strain; P = susceptible strain.

histamine mepyramine maleate (5·0 mg/kg intramuscularly 30 min before histamine) reduces ulceration by 50% ($P<0·01$), the only change in the secretory pattern being an increase ($P<0·01$) in the volume of gastric juice secreted.

Discussion

The technique described will yield consistent gastric ulceration in a susceptible strain of guinea-pig. Histamine is required in relatively low dosage of aqueous solution injected subcutaneously and this contrasts with the larger doses of longer acting non-aqueous suspensions used in earlier methods. By this present method, the gastric response is likely to be more uniform, since several complicating factors are eliminated. Where delayed absorption is obtained by intramuscular injection of suspensions containing relatively high doses of histamine and of varying physico-chemical properties, the different responses obtained, for example by Olovson (1950) and Williams (1951), probably indicate varying release rates from the sites of injection. By our technique these factors are

minimised. An aqueous solution of histamine acid phosphate is more likely, after relatively rapid absorption, to yield a threshold histamine level more quickly and more certainly than an oily suspension which after slower absorption would give a lower, more prolonged but less consistent level; and it may well be that the ulceration and secretory effects here measured after 1 hr, follow such a level more consistently. This may explain one of our unreported findings that the severity of gastric ulceration was variable when such a suspension was given by the intramuscular route but not so with the present technique.

The finding that urethane was not a suitable anaesthetic bears out Schachter's (1949) findings that urethane stimulated secretion of high acidity in dogs after a delay of 2 hr. These effects may be referable to a stimulating effect of urethane on the secretion of endogenous histamine.

The difference in resistance between the two strains of guinea-pig therefore appears to be to ulceration rather than to changes in secretory pattern, which are broadly similar; whether this is an intrinsic difference between strains in terms of gastric response to histamine or whether there is some other factor is not yet clear. This uniformity of secretion accompanying a varying ulcer-proneness is of direct interest when considering the relationship between results obtained using this animal and their relevance to the disease in man where ulcer-proneness is an accepted though not a clearly understood concept.

In the guinea-pig, histamine ulceration has been variously attributed either to the secreted gastric juice (Hay & others, 1942; Halpern & Martin, 1946; Zaidi & Mukerji, 1958) or to the angiotoxic effect of histamine (Merkel, 1942; Williams, 1951; Kowalewski, 1954) and recently Watt (1959) has suggested that the ulcerative process is initiated in the first instance by an effect of the acid gastric juice on the mucosal vessels. The present results indicate that, in the guinea-pig, whether or not the particular strain is ulcer-resistant, an increase in histamine dosage beyond a certain level results in an output of gastric juice lower than that produced by doses not producing ulceration, an observation made under different experimental conditions by Ivy, Grossman & Bachrach (1951). These results are interpreted as indicating that at the higher (ulcerogenic and above) dose of histamine, a depressant effect on the cellular function of the secreting mucosa sets in as evidenced by failure of secretion volume to increase with dose, and this could be indirectly referable to the angiotoxic effects of histamine, whereas below this ulcerogenic threshold, the action of histamine on the secretory function appears to be one of increasing stimulation.

In the ulcer-prone strain, this is sufficient to allow the juice secreted in lower volume to take its part in the ulcerative process. The necessity of secreted acid is borne out by the results of the experiments with aluminium hydroxide where complete protection against ulceration is found with a complete neutralisation of free acidity of the gastric secretion. The results of the experiments are therefore compatible with the concept that both the angiotoxic effects as well as irritant action of hydrochloric acid are concerned in ulcerogenesis under these conditions.

Antihistamines have frequently been used in studies on the experimental ulcer ever since Halpern & Martin (1946) made it known that a potent antihistamine will protect the guinea-pig against systemic effects of histamine given in a dosage sufficient to produce peptic ulceration at the same time. We find that the potent antihistamine promethazine does modify the ulceration picture when a sub-lethal dose of histamine is used as the ulceration stimulus.

Unlike mepyramine maleate, the anti-ulcer property of promethazine appears not to be associated with increase in volume of secretion. The differences in the effects of the different antihistamines is not surprising since pharmacological experience reveals that specificity of action is relative and not absolute, absolute specificity being rare or practically unattainable (Loew, 1947). Promethazine, besides being an antihistamine, exhibits atropine-like and ganglion blocking action and with these actions secretion volume is unchanged, and ulceration fails to occur. Mepyramine maleate, while being more specific, is a weaker antihistamine than promethazine, and its ulcer-protective effect is accompanied by an increase in volume of secretion. Wood (1949) also observed an increase in volume of secretion after mepyramine in histamine-stimulated cats. The increased volume of secretion and the diminution of ulceration may well be due to improved circulation in the gastric vascular bed, as a result of histamine antagonism.

This work, while providing a method of producing gastric ulcers in the guinea-pig, also supports the view that histamine gastric ulceration in the guinea-pig is apparently the result of the action of gastric juice on a mucosa functionally disturbed by histamine.

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