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PHENYLBUTAZONE AND HISTAMINE FORMATION IN RAT GLANDULAR STOMACH: ITS RELATIONSHIP TO GASTRIC ULCERATION

BY

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It is well known that large doses of phenylbutazone (Butazolidine) will produce gastric ulceration in humans as well as in animals. Bonfils, Hardouin & Bourel (1953), were the first to use phenylbutazone for the promotion of ulceration in rats, and since then both they and other workers have used the drug for this purpose (Bonfils, Hardouin & Delbarre, 1954 ; Bonfils, Liefooghe, Rossi and Lambling, 1957 ; Levrat & Lambert, 1959 ; Brodie, Marshall & Moreno, 1962 ; Schwartz, Cohen & Vallete, 1966 ; Abdel-Galil, 1967).

The mechanism by which phenylbutazone produces this ulcerogenic effect is, however, not yet clear. Kirsner (1957) thought that decreased tissue resistance in the presence of hydrochloric acid was the aetiological mechanism for ulceration. Segal (1960) suggested a possible role played by injury to parietal cells by phenylbutazone which produced a cellular toxic effect, reducing the tissue resistance to digestion. Brodie *et al.* (1962) could not find any apparent relationship between acid secretion and phenylbutazone ulceration, which contradicts the finding of Bonfils *et al.* (1954), who observed a reduction in gastric secretion in rats treated with phenylbutazone and postulated a direct toxic action for the drug. Our preliminary experiments showed that intramuscular injection of phenylbutazone into rats was followed by a sharp rise in urinary free histamine excretion, which was subsequently found to originate from increased histamine formation in the gastric mucosa. The experiments reported here were designed to investigate to what extent this effect was under hormonal control, and whether the increased formation of histamine or its release from the glandular stomach had any relationship to the ulcerogenic action of the drug.

METHODS

Female rats of the Wistar strain, weighing 100–350 g, were used throughout. Operations were done under ether anaesthesia with antiseptic precautions. Urine samples were collected from groups of rats housed in circular metabolic cages (Marshall, 1961). Acidified 24 hr urine specimens were stored at 4° C until assayed for histamine.

Adrenalectomy was carried out by the bilateral routes of approach. Adrenalectomized rats were maintained on a normal diet and given 0.9% (w/v) sodium chloride solution to drink.

Hypophysectomy was performed by the ventral approacn ; the trachea was deflected to clear the base of the skull by pushing back the muscle attachments with small cotton-wool swabs. The skull

was drilled with a No. 10 dental burr at the junction of the midline with the spheno-occipital suture and the pituitary gland was sucked out through a fine glass tube attached to a water pump. The animals were maintained on normal diet and drinking water after the operation.

Thyroidectomy was carried out through a ventral midline incision in the neck. Each lobe of the gland was removed separately, after division of the connecting isthmus. The animals were given 1% (w/v) calcium gluconate in 10% (w/v) glucose solution to drink for the first 24 hr after operation, but no food. Thereafter, they received normal diet and 1% (w/v) calcium gluconate to drink. The calcium gluconate was given to counteract any effects of parathyroid deficiency.

Selective bilateral vagotomy (subsequently referred to as vagotomy) was carried out as described by Lambert (1965). The left vagus was dissected out for a distance of 15 mm below the diaphragm and a segment 10 mm long removed. The bundle of tisuse between the left gastric artery and the right border of the oesophagus, including the peritoneal serous layer, the right vagus trunk and a little fatty tissue, but excluding the left gastric artery itself, was dissected out for a distance of 10–15 mm. This bundle of tissue was then divided below the diaphragm and a segment 10 mm long removed. Finally, the oesophagus was stripped of any associated tissue or nerve fibres in order to ensure complete vagal denervation of the stomach. Post-operatively the animals were given 25% (w/v) glucose solution to drink for the first 24 hr ; thereafter they received normal diet and water.

Double operative procedures were carried out, in most cases during the same period of anaesthesia. These included adreno-thyroidectomy, adreno-vagotomy and thyro-vagotomy.

In all experiments involving operative procedures, control groups of rats were sham-operated by carrying out the appropriate operative techniques, without removal of the particular gland or tissue.

Histamine forming capacity (H.F.C.) was measured in the glandular region of the rat stomach as already described (Abdel-Galil & Marshall, 1967), by incubating a centrifuged homogenate of the tissue with L-histidine (2.0 mg/ml.) for 3 hr at 37° C in phosphate buffer of pH 7.2. The tissue, dissected from the animal killed by a blow on the head, or within 30 min of death, was weighed, ground with sand and suspended in such a quantity of ice-cold 0.9% sodium chloride solution that 5 ml. of suspension contained 1 g of tissue. The suspension was centrifuged for 15 min at 3,000 rev/min (M.S.E. Major centrifuge), and the supernatant fluid used for the incubation. In some experiments, mucosa scraped from the muscular layers was used in the same proportion of 1 g of original tissue to 5 ml. of fluid. There was no difference in H.F.C. whichever preparation was used, which indicates that the whole activity was within the mucosa.

Histamine from urine and the incubates of gastric tissue, with or without the addtion of L-histidine, was estimated biologically on the guinea-pig ileum suspended in atropinized Tyrode solution by the 4-point assay design in the automatic organ bath of Boura, Mongar & Schild (1954). Specificity of the gut-contracting substance was checked with mepyramine maleate as described by Reuse (1948).

The ulceration index in rat stomach was estimated by the method of Pauls, Wick & McKay (1947). The degree of ulceration was assessed by observation of the mucosa of the opened glandular portion of the stomach and scores allotted as follows:

Normal stomach	0
Petechial haemorrhage, or pin-point ulcers	1
One or two small ulcers	2
Many ulcers, a few large	3
Many ulcers, mainly large	4

The ulceration index was then obtained by multiplying the degree of ulceration in a group of rats by the percentage of animals showing ulceration.

Drugs. Phenylbutazone, supplied by Geigy Ltd., Manchester, was prepared for injection either as a suspension of 1 g in 20 ml. of mucilage of tragacanth, or as a solution of the sodium salt prepared by addition of 0.1 N sodium hydroxide, and used in a concentration of 1 g/20 ml. (pH 8–9). KB95, (2-(*N'*-methyl piperid-4'yl)4-benzyl, 5 phenyl-3-oxo-pyrazoline) supplied by Sandoz Ltd., Basle, was used in a concentration of 1 g/20 ml. water and sodium salicylate in a concentration of 1 g/20 ml. water.

RESULTS

Effect of phenylbutazone on urinary free histamine excretion

Two groups, each of three female rats (150 g weight), were kept in metabolic cages and serial 24 hr urine specimens collected. On the fourth day one group of rats received phenylbutazone 100 mg/kg in mucilage of tragacanth, injected into the thigh muscle. The other group received a similar volume of mucilage of tragacanth intramuscularly. This treatment caused a sharp rise in histamine excretion in both groups (Fig. 1), but

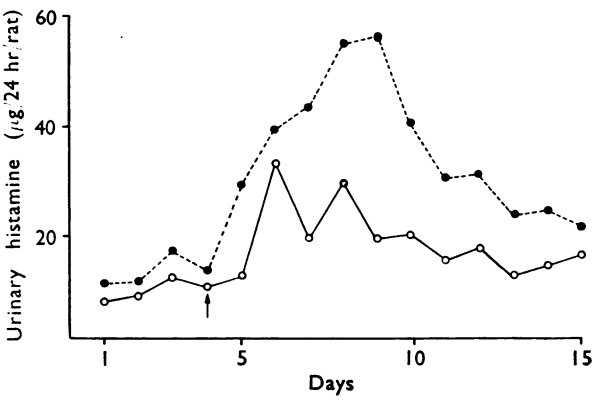

Fig. 1. Effect of intramuscular injection of phenylbutazone on the excretion of free histamine in the urine of female rats. At arrow, injection of phenylbutazone 100 mg/kg, ● or mucilage of tragacanth 2 mg/kg, ○.

that in the phenylbutazone group was significantly higher ($P<0.01$) than in the control group. A dose of phenylbutazone 200 mg/kg produced an increase in histamine excretion significantly greater than that caused by 100 mg/kg ($P=0.001$). Doses higher than 200 mg/kg were toxic. A dose of phenylbutazone 50 mg/kg daily for 15 days produced an effect on histamine excretion not significantly greater ($P=0.2$) than that produced by equivalent injections of mucilage of tragacanth. Single doses of phenylbutazone 200 mg/kg were therefore used in all the experiments on gastric H.F.C.

Effect of phenylbutazone on H.F.C. and ulceration index

Normal rats

Identical results were obtained with both the phenylbutazone suspension and its solution as sodium salt, and in the following experiment the solution of sodium phenylbutazone was used. A total of 126 female rats of Wistar strain weighing 250 ± 25 g were divided into seven groups, each of eighteen rats, having approximately the same total body weight. Each group of eighteen rats was starved overnight and then divided into sub-groups which were treated as follows: six rats received a single dose of phenylbutazone 200 mg/kg given by the intramuscular route ; six rats received a single dose of 0.9% sodium chloride solution 4 ml./kg adjusted to pH 8.0–9.0 with 0.1 N sodium hydroxide, given intramuscularly ; six rats were given no treatment at all.

Environmental disturbances were reduced to a minimum after the injections in order to avoid external stress. Groups of eighteen rats were killed at increasing intervals from the time of injection. The stomachs were removed, assessed for degree of ulceration, and homogenates of the glandular portions prepared for estimation of endogenous histamine content and histamine forming capacity.

The results are shown in Fig. 2 and Table 1. The groups of rats which received phenylbutazone and were killed 6 hr after injection showed the highest values of ulceration index and the highest increase in H.F.C. above that of the saline group (Table 1). No significant difference was found between the H.F.C. and endogenous histamine levels of the groups receiving saline and those receiving no treatment. Injection of 0.9% sodium chloride solution produced no observable change in the appearance of the glandular stomach, while phenylbutazone produced all four degrees of ulceration and a significant increase in H.F.C.

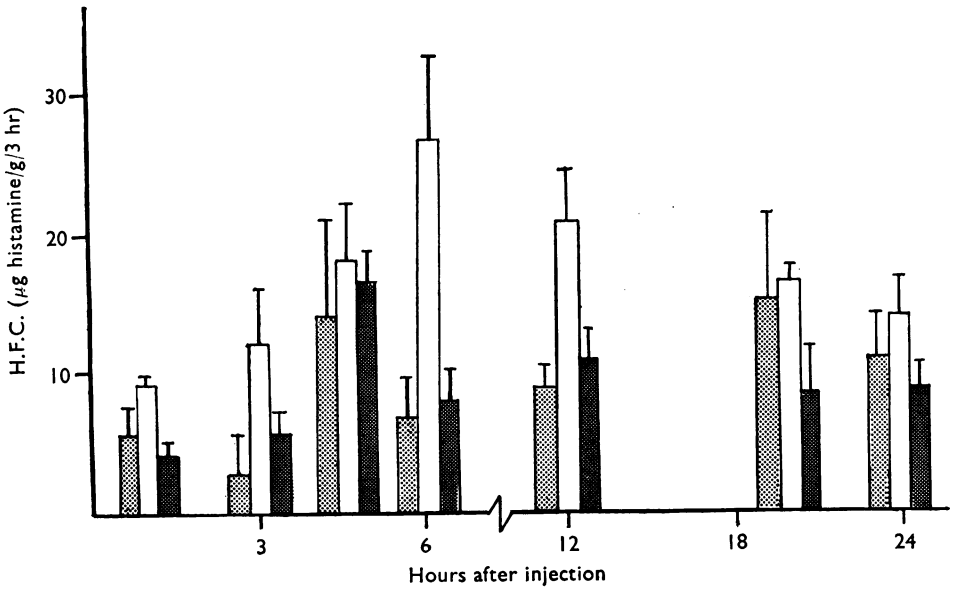

Fig. 2. Effect of intramuscular injection of phenylbutazone (200 mg/kg) and 0.9% (w/v) sodium chloride solution (4 ml./kg) on histamine forming capacity (H.F.C.) of the glandular stomach of female rats. In each group of columns, the first indicates the mean value obtained from six untreated rats, the second the mean value from six rats injected with phenylbutazone and the third the mean value from six rats injected with 0.9% sodium chloride solution. The vertical bars above the columns are S.E. of the means.

TABLE 1

EFFECT OF INTRAMUSCULAR INJECTION OF PHENYLBUTAZONE (200 mg/kg) ON GASTRIC ULCERATION AND HISTAMINE FORMING CAPACITY (H.F.C.) OF GASTRIC MUCOSA OF NORMAL RATS

Each value is the mean obtained from six female rats. * H.F.C. of rats injected with phenylbutazone compared with rats injected with 0·9% sodium chloride solution. † H.F.C. of rats injected with phenylbutazone compared with untreated rats.

Time after injection (hr)	Rats showing ulceration (%)	Ulceration index	Increase in H.F.C. above level of saline group (%)	P_1*	P_2†
1	16·7	16·7	154·0	0·01	0·1
3	66·7	133·4	115·4	0·1	0·05
4½	66·7	200·1	9·9	0·7	0·4
6	83·3	249·9	235·0	0·01	0·01
12	66·7	200·1	93·6	0·02	0·01
20	66·7	133·4	103·0	0·02	0·8
24	50	100	55·3	0·1	0·4

Adrenalectomized rats

Eighteen female rats weighing 200±25 g were divided into two groups of nine rats each. Rats of one group were bilaterally adrenalectomized while those of the other group were sham-operated. Nineteen days after operation the rats were fasted overnight for 8 hr, and then each was given a single intramuscular injection of phenylbutazone 200 mg/kg.

Ninety minutes after phenylbutazone injection adrenalectomized rats showed signs of histamine poisoning, including slow and difficult respiration and flushing of the extremities. Two hours after injection, some rats showed mild repeated clonic convulsions of 10–15 sec duration, lasting for a period of 90 min. Three hours after injection some of the adrenalectomized rats died in a state of convulsion and at the end of 4 hr after injection all adrenalectomized rats were dead. None of the sham-operated rats died within this 4 hr period, but they were then killed to be used as controls. At autopsy adrenalectomized rats had a large quantity of blood in both small and large intestine, and blood was always found in the stomach contents. Sham-operated rats had no blood in the stomach, small or large intestine.

There was no significant difference in H.F.C. level or endogenous histamine content between sham-operated and adrenalectomized rats treated with phenylbutazone (Table 2). All the adrenalectomized rats showed third degree ulceration. The death of these rats was most probably caused by blood loss by haemorrhage into the stomach and extreme congestion of the abdominal viscera.

TABLE 2

EFFECT OF INTRAMUSCULAR INJECTION OF PHENYLBUTAZONE (200 mg/kg) ON HISTAMINE FORMING CAPACITY (H.F.C.), ENDOGENOUS HISTAMINE OF THE STOMACH, GASTRIC ULCERATION AND MORTALITY RATE IN ADRENALECTOMIZED RATS

Each value is the mean ± S.E. obtained from nine female rats.

	Adrenalectomized rats	Sham-operated rats	*P*
H.F.C. (μg/g, 3 hr)	4·8±1·1	7·7±1·4	0·1
Endogenous histamine content of the stomach (μg/g)	14·6±1·7	16·5±1·7	0·4
Degree of ulceration	3	1 ±0·3	0·001
Ulceration index	300	66·7	
Mortality rate 4 hr after phenylbutazone injection (%)	100	0	

When adrenalectomized rats were fasted for 24 hr before administration of phenylbutazone, the degree of ulceration was increased to the maximum; all adrenalectomized rats died while there were no deaths in the sham-operated group.

Hypophysectomized rats

Twenty female rats weighing 200±10 g were used. Ten rats were hypophysectomized and ten sham-operated. Six weeks after operation, the rats were fasted for 24 hr, and divided into four groups, each of five rats. Two groups, one hypophysectomized and one sham-operated, received a single intramuscular injection of phenylbutazone 200 mg/kg. The remaining two groups, one hypophysectomized and one sham-operated, received a single intramuscular injection of 0.9% sodium chloride solution 4 ml./kg, pH 8–9.

Two hours after injection some hypophysectomized rats showed signs of histamine poisoning. Four of the hypophysectomized rats died 2–3 hr after injection of phenylbutazone; blood was found in the small intestine and stomach. The remaining rats were killed 6 hr after injection. Only one of the sham-operated rats injected with phenylbutazone had blood in its stomach. Hypophysectomy did not produce any significant change in H.F.C. or endogenous histamine content of the rat stomach (Table 3).

TABLE 3

EFFECT OF INTRAMUSCULAR INJECTION OF PHENYLBUTAZONE (200 mg/kg) ON HISTAMINE FORMING CAPACITY (H.F.C.) AND ENDOGENOUS HISTAMINE OF THE STOMACH, GASTRIC ULCERATION AND MORTALITY RATE IN HYPOPHYSECTOMIZED RATS

Each value is the mean ± S.E. obtained from five female rats. * 0·9% sodium chloride solution of pH 8–9 (4 ml./kg)

	Drug administered intramuscularly	Hypophysectomized rats	Sham-operated rats	*P*
H.F.C. (μg/g, 3 hr)	Saline*	5·7±2·1	3·5±2·9	0·6
Endogenous histamine content of the stomach (μg/g)	Saline*	19·3±5·1	14·3±4·4	0·5
H.F.C. (μg/g, 3 hr)	Phenylbutazone	13·0±3·8	18·9±3·6	0·4
Endogenous histamine content of the stomach (μg/g)	Phenylbutazone	22·3±3·7	13·3±4·4	0·1
Mortality rate 6 hr after injection (%)	Phenylbutazone	80	0	
Degree of ulceration	Phenylbutazone	2·6±0·6	2·4±0·8	0·8
Ulceration index	Phenylbutazone	260	192	

Hypophysectomized rats injected with phenylbutazone did not have convulsions similar to those seen in adrenalectomized rats after phenylbutazone. Moreover, hypophysectomy did not affect the ulcerogenic action of phenylbutazone.

Vagotomized rats

Two groups, each of twelve rats weighing 200±10 g, were used in this experiment. One group was bilaterally vagotomized and the other was sham-operated. The rats were kept on normal rat cake and water for 35 days after operation, and then fasted for 24 hr. Six rats from each group were given an intramuscular injection of sodium phenylbutazone 200 mg/kg while the rest of the rats were given an intramuscular injection of 0.9% sodium chloride solution 4 ml./kg, pH 8–9. None of the rats which received phenylbutazone showed any abnormal symptoms and all were killed 6 hr after injection. There was no significant difference between endogenous histamine content and the H.F.C. of vagotomized and sham-operated rats which received phenylbutazone. Vagotomy decreased the degree of ulceration in rats receiving phenylbutazone (Table 4).

Effect of adrenalectomy in vagotomized rats

Twelve female rats were vagotomized; they were kept on normal rat cake and water for 21 days after operation and were then divided into three groups, each of four rats. Group 1 was adrenalectomized and groups 2 and 3 were sham-operated. Thirty-five days after adrenalectomy the rats were fasted overnight. Groups 1 and 2 received a single intramuscular injection of phenylbutazone (200 mg/kg). Group 3 received a single intramuscular injection of 0.9% sodium chloride solution 4 ml./kg, pH 8–9. Three rats in the group wih vagotomy and adrenalectomy died 3.5 hr after injection of phenyl-

TABLE 4

EFFECT OF INTRAMUSCULAR INJECTION OF PHENYLBUTAZONE (200 mg/kg) ON HISTAMINE FORMING CAPACITY (H.F.C.) AND ENDOGENOUS HISTAMINE OF THE STOMACH, GASTRIC ULCERATION AND MORTALITY RATE IN VAGOTOMIZED RATS

Each value is the mean ± S.E. obtained from six female rats. * 0·9% sodium chloride solution of pH 8–9 (4 ml./kg)

	Drug administered intramuscularly	Vagotomized rats	Sham-operated rats	*P*
H.F.C. (μg/g, 3 hr)	Saline*	8·3±2·0	4·3±3·2	0·3
Endogenous histamine of the stomach (μg/g)	Saline*	19·7±2·4	21·3±1·2	0·6
Ulceration index	Saline*	0	0	
H.F.C. (μg/g, 3 hr)	Phenylbutazone	20·8±4·8	23·4±10·4	0·8
Endogenous histamine of the stomach (μg/g)	Phenylbutazone	36·0±5·1	27·6±2·1	0·1
Degree of ulceration	Phenylbutazone	1 ±0·4	2·3±0·3	0·05
Ulceration index	Phenylbutazone	66·7	233·3	
Mortality rate 6 hr after injection (%)	Phenylbutazone	0	0	

butazone. The remaining rats were killed 4 hr after injection. In vagotomized rats, adrenalectomy increased both the number of rats showing ulceration and the ulceration index. The degree of ulceration was possibly more severe but the difference was not significant. Adrenalectomy increased the mortality rate of vagotomized rats ; none of the rats which died after injection of phenylbutazone had blood in the small intestine. Adrenalectomy had no significant effect on H.F.C. or endogenous histamine levels in vagotomized rats injected with phenylbutazone (Table 5).

TABLE 5

EFFECT OF INTRAMUSCULAR INJECTION OF PHENYLBUTAZONE (200 mg/kg) ON HISTAMINE FORMING CAPACITY (H.F.C.) AND ENDOGENOUS HISTAMINE OF THE STOMACH, GASTRIC ULCERATION, AND MORTALITY RATE OF RATS WHICH HAVE BEEN VAGOTOMIZED FOR 21 DAYS AND THEN ADRENALECTOMIZED FOR 35 DAYS

Each value is the mean ± S.E. obtained from four female rats.

	Vagotomized and adrenalectomized rats injected with phenylbutazone	Vagotomized rats injected with phenylbutazone	Vagotomized rats injected with 0·9% NaCl solution of pH 8–9 (4 ml./kg)	*P* (Vagotomy *v.* vagotomy plus adrenalectomy)
H.F.C. (μg/g, 3 hr)	7·3±1·4	14·0±7·5	9·2±2·0	0·4
Endogenous histamine content of the stomach (μg/g)	22·5±3·1	13·8±4·7	22·3±2·2	0·2
Degree of ulceration	2±0	1±0·4	0	0·1
Ulceration index	200	75	0	
Mortality rate 4 hr after injection (%)	75	0	0	

Thyroidectomized rats

Eight female rats weighing 200 ± 10 g were thyroidectomized. Twenty-eight days after operation the rats were fasted for 24 hr ; four rats received a single intramuscular injection of phenylbutazone 200 mg/kg and four received 0.9% sodium chloride solution 4 ml./kg, pH 8–9. One rat from the group which received phenylbutazone died 5 hr after injection and at autopsy blood was found in the stomach and small intestine. The remaining rats were killed 6 hr after injection ; only one of these animals had blood in the small intestine. Phenylbutazone produced a significant increase in H.F.C. in thyroidectomized rats (Table 6). Thyroidectomy did not protect rats from developing gastric ulceration

TABLE 6

EFFECT OF INTRAMUSCULAR INJECTION OF PHENYLBUTAZONE (200 mg/kg) ON HISTAMINE FORMING CAPACITY (H.F.C.) AND ENDOGENOUS HISTAMINE OF THE STOMACH, GASTRIC ULCERATION AND MORTALITY RATE ON THYROIDECTOMIZED RATS

Each value is the mean ± S.E. obtained from groups of four female rats.

	Thyroidectomized rats injected with phenylbutazone	Thyroidectomized rats injected with 4 ml./kg 0·9% NaCl solution of pH 8–9	*P*
H.F.C. (μg/g, 3 hr)	28·3±10·8	5·2±1·8	0·05
Endogenous histamine content of the stomach (μg/g)	20·9±2·4	16·0±3·8	0·4
Mortality rate 6 hr after injection (%)	25	0	
Ulceration index	200	0	

after phenylbutazone injection, but the ulceration index of thyroidectomized rats (200) was lower than that of normal rats under similar treatment (249.1) and much lower than that of adrenalectomized rats (400). The mortality rate after phenylbutazone administration was lower in thyroidectomized than in adrenalectomized rats.

Double-operated rats

The following groups of four female rats each were used in this experiment: Group 1, controls; group 2, adrenalectomized and sham-operated for thyroidectomy; group 3, thyroidectomized and sham-operated for adrenalectomy; group 4, sham-operated for adrenalectomy and thyroidectomy; group 5, thyroidectomized and adrenalectomized; group 6, vagotomized and thyroidectomized. Forty-one days after operation the rats were fasted for 24 hr.

The rats in groups 1, 2, 3, 5 and 6 were given a single intramuscular injection of phenylbutazone 200 mg/kg. The rats in group 4 were given a single intramuscular injection of 0.9% sodium chloride solution 4 ml./kg, pH 8–9. Blood was found in the stomachs and small intestines of all rats which died after injection of phenylbutazone. Thyroidectomized and vagotomized rats (group 6) and normal rats (group 1) injected with phenylbutazone had a higher level of H.F.C. than the double-sham-operated rats (group 4) which received alkaline saline ($P=0.05$). Thyroidectomized and adrenalectomized rats (group 5) had the same ulceration index as adrenalectomized rats (group 2) but only half their mortality rate. Thyroidectomized and vagotomized rats (group 6) had a lower ulceration index than thyroidectomized rats (group 3) but had a higher mortality rate. Thyroidectomized and adrenalectomized rats (group 5) showed a higher degree of ulceration than thyroidectomized and vagotomized rats (group 6) but there was no difference in mortality rates (Table 7).

Effect of KB95 and sodium salicylate on H.F.C. and ulceration index

The effect of phenylbutazone was compared with the effects of the closely related KB95 and of sodium salicylate.

Four groups, each of six female rats, weighing 150 ± 10 g, were used in this experiment. They were fasted for 24 hr and were then given single intramuscular injections as follows: group 1, phenylbutazone 200 mg/kg (5% w/v solution); group 2, KB95 200 mg/kg (5%

TABLE 7

EFFECT OF INTRAMUSCULAR INJECTION OF PHENYLBUTAZONE (200 mg/kg) ON HISTAMINE FORMING CAPACITY (H.F.C.) ENDOGENOUS HISTAMINE CONTENT OF THE STOMACH, GASTRIC ULCERATION AND MORTALITY RATE ON DOUBLE OPERATED RATS

* 1, Controls; 2, adrenalectomized and sham-operated for thyroidectomy; 3, thyroidectomized and sham-operated for adrenalectomy; 4, sham-operated for adrenalectomy and thyroidectomy; 5, thyroidectomized and adrenalectomized; 6, vagotomized and thyroidectomized. All rats in group 2 died between 1·5 and 5 hr after injection of phenylbutazone, one rat from group 3 after 5 hr, two rats from group 5 after 3·5 hr, two rats from group 6 after 4·5 hr; the remaining rats were killed after 6 hr. † 0·9% sodium chloride solution of pH 8–9 (4 ml./kg).

Groups of four female rats*	Drug administered intramuscularly	H.F.C. (μg/g, 3 hr)	Endogenous histamine content of the stomach (μg/g)	Degree of ulceration	Ulceration index	Mortality rate 6 hr after injection (%)
1	Phenylbutazone	14·9±2·5	15·4± 1·0	2·5±0·6	250	0
2	Phenylbutazone	5·3±2·1	15·8± 1·2	4	400	100
3	Phenylbutazone	7·6±2·8	15·0± 4·3	2·3±0·8	225	25
4	Saline†	5·5±2·1	13·0± 4·0	0	0	0
5	Phenylbutazone	7·6±0·8	10·3± 3·4	4	400	50
6	Phenylbutazone	65·8±23·2	47·6±12·0	1·8±0·3	175	50

w/v solution); group 3, sodium salicylate 200 mg/kg (5% w/v solution); group 4, 0.9% sodium chloride solution 4 ml./kg. The rats were killed 6 hr after injection.

All three anti-inflammatory drugs produced significant increases in H.F.C. above the value found in the saline-treated controls. On the other hand, the ulceration index after phenylbutazone was very high, that with sodium salicylate much lower, while KB95 produced no ulceration at all (Table 8).

TABLE 8

EFFECT OF INTRAMUSCULAR INJECTION OF PHENYLBUTAZONE (200 mg/kg), KB95 (200 mg/kg) AND SODIUM SALICYLATE (200 mg/kg) ON HISTAMINE FORMING CAPACITY (H.F.C.) AND ENDOGENOUS HISTAMINE CONTENT OF THE STOMACH AND GASTRIC ULCERATION ON NORMAL RATS

* 0·9% sodium chloride solution of pH 8–9 (4 ml./kg). † H.F.C. of saline-treated group compared with groups treated with phenylbutazone, KB95 or sodium salicylate.

Each value is the mean ± S.E. obtained from groups of six female rats.

Drug administered	H.F.C. (μg/g, 3 hr)	Endogenous histamine content of the stomach (μg/g)	Ulceration index	P†
Phenylbutazone	4·5±0·8	12·4±2·2	250	0·02
KB95	4·6±0·1	11·0±1·4	0	0·001
Sodium salicylate	6·6±0·8	13·5±1·9	50	0·001
Saline*	1·8±0·9	12·6±0·5	0	

Effect of phenylbutazone, KB95 and sodium salicylate on H.F.C. of rat glandular stomach in vitro

Rat glandular stomach homogenate was prepared from pooled tissues from twenty female rats. Incubation mixtures for the determination of H.F.C. and endogenous histamine were prepared in the usual manner, except that 0.12 ml. of the distilled water, used to make up the volume of the incubation mixture to 3 ml. was replaced by 0.12 ml. of a solution containing 24 μg of the drug to be tested, because with a dose of 200 mg/kg, 0.12 g of tissue would contain about 24 μg of drug. All incubations were done in

duplicate and the means of five different experiments are recorded in Table 9. Addition of these drugs to the incubation mixture did not significantly change the H.F.C. or endogenous histamine.

TABLE 9

EFFECT OF PHENYLBUTAZONE, KB95 AND SODIUM SALICYLATE ON HISTAMINE FORMING CAPACITY (H.F.C.) OF RAT GLANDULAR STOMACH TISSUE HOMOGENATES (*IN VITRO*)

Drugs (24 μg/0·12 ml. of H_2O) were added to incubation mixture of 3 ml. final volume. Each value is the mean ± S.E. obtained from five experiments, each of which was done in duplicate. * H.F.C. of incubation mixture without drug compared with mixture containing drugs.

Drugs added	H.F.C. (μg/g, 3 hr)	Endogenous histamine content of the tissue homogenate (μg/g)	*P**
Control	7·9±4·0	26·3±5·7	0·4
Phenylbutazone	13·0±6·0	19·1±5·9	0·3
KB95	13·7±4·9	34·1±6·2	0·5
Sodium salicylate	12·8±6·6	28·4±3·7	

DISCUSSION

Administration of phenylbutazone in a dose of 200 mg/kg body weight to rats fasted overnight was found to produce, in the glandular stomach, an increase in histamine formation associated with gastric ulceration in the same region. This response reached a peak 6 hr after injection.

This increase in histamine formation could be responsible for the development of gastric ulceration because histamine has been considered as a contributing agent in the production of the aggressive acid-pepsin factor (Emmelin & Kahlson, 1944; Code, 1956, 1965; Kahlson, Rosengren, Svahn & Thunberg, 1964; Haverback, Stubrin & Dyce, 1965; Fischer & Snyder, 1965; Levine, 1965; Ivy & Bachrach, 1966; Beaven, Harakova, Johnson, Erjavec & Brodie, 1967). Moreover, histamine has also been thought to take part in the regulation of the gastric microcirculation (Schayer, 1962a, 1963, 1964, 1965, 1966). The observed relationship between ulceration and increased histamine formation did not hold, however, when rats were injected with sodium salicylate or KB95 which is structurally related to phenylbutazone. Although all three drugs increased histamine formation, there was a large increase in ulceration index only in the rats which received phenylbutazone.

None of these drugs caused a significant change in histamine formation *in vitro* when used in concentrations comparable with those calculated to occur in the *in vivo* experiments. The stimulation of histamine formation is therefore a true *in vivo* effect on the specific histidine decarboxylase of the gastric mucosa.

Only phenylbutazone produced gastric ulceration, so the increased histamine formation cannot alone be responsible for the ulcerogenic action of this drug. For this reason, the influence of the endocrine glands was studied, because these are known to produce selective adaptive changes on the rat glandular stomach (Schayer, 1957, 1960; Abdel-Aziz, 1963; High, Shepherd & Woodcock, 1965; Abdel-Galil, 1967). Administration of phenylbutazone increased histamine formation in both adrenalectomized and sham-operated rats to the same degree, but adrenalectomized rats were found to be more

sensitive to the increased histamine formation and died within 3–4 hr of phenylbutazone injection. At death there was extreme congestion of the abdominal viscera, and haemorrhage into the small intestine; the ulceration index was increased. The sensitivity of adrenalectomized rats to phenylbutazone was increased by fasting for 24 hr before administration of the drug; this produced a further increase in the ulceration index and a decrease in the survival time after injection. An increased sensitivity of adrenalectomized rats to histamine is well known (Voegtlin & Dyer, 1924; Crivellari, 1927; Marmorston-Gottesman & Gottesman, 1928; Scott, 1928; Perla & Marmorston-Gottesman, 1931; Noble & Collip, 1941). There is some evidence that the increased histamine formation *in situ* (Schayer, 1962b) and the diminished resistance of the atrophied gastric mucosa in the adrenalectomized rats (Shay, Bralow, Kovarov & Kessler, 1962; Kyle, Clarke, Ward, Adesola & Welbourn, 1963) are responsible for the development of ulceration and death in these animals.

The close relationship between the adrenals and the pituitary in the rat is well documented. Schayer (1957) showed that adrenalectomy and hypophysectomy produced similar effects on *in vivo* formation and binding of ^{14}C-histamine in the rat. In the present work, the sensitivity of hypophysectomized rats to phenylbutazone was similar to that found in adrenalectomized rats although the ulceration index and the mortality rate were slightly lower in hypophysectomized rats. The increased sensitivity of these rats to the drug may have resulted from their reduced general resistance, because they lost 30–50 g body weight during the experimental period.

Thyroid hormones are known to influence gastric secretion and histamine formation in rats. Nasset, Logan, Kelley & Thomas (1957, 1959) and Goldsmith & Nasset (1959) found that feeding of desiccated thyroid decreased gastric secretion, but, on the other hand, Sun, Shay, Siplet & Grunstein (1954) and Goldsmith & Nasset (1959) showed that thyroidectomy also depressed gastric secretion. Abdel-Galil (1967) showed that thyroid hormone, administered intraperitoneally, increased gastric histamine formation while thyroidectomy reduced it. Administration of phenylbutazone to thyroidectomized rats caused increased histamine formation and gastric ulceration; however, the degree of ulceration was lower in thyroidectomized than in sham-operated rats.

In vagotomized rats, administration of phenylbutazone resulted in a lower ulceration index than in sham-operated rats, although the increase in gastric histamine formation was the same in both groups. This protective effect of vagotomy against ulceration may well be due to an increase in the defensive factors of the gastric mucosa, because vagotomy causes improvement in the mucosal blood supply (Nylander & Olerud, 1961) and an increase in mucosal weight (Dorchester, 1959).

The protective action of vagotomy was also present in adrenalectomized rats. The ulceration index of adrenalectomized rats which had also been vagotomized was higher than that of vagotomized rats, but lower than that of adrenalectomized rats.

In adrenalectomized rats which had also been thyroidectomized, phenylbutazone caused an ulceration index which was similar to that in adrenalectomized rats with the thyroid intact and there was no change in histamine formation or histamine content of the glandular stomach. On the other hand, thyroidectomy reduced the lethal effects of phenylbutazone in adrenalectomized rats.

The results presented in this paper suggest that phenylbutazone has a depressant effect on the mucosal defensive factor of the rat gastric mucosa ; this effect can be modified by changes in the hormonal balance of the animal. On the other hand, the increased histamine formation in the glandular mucosa suggests that phenylbutazone also affects aggressive factors, particularly the acid-pepsin factor.

SUMMARY

1. The mechanisms by which phenylbutazone produces its ulcerogenic effects in the rat glandular stomach were investigated. Intramuscular administration of phenylbutazone (200 mg/kg) to rats fasted overnight increased histamine formation and produced ulceration of the glandular stomach ; maximum effects were reached after 6 hr. KB95 (2-(*N'*-methyl piperid-4'yl)4-benzyl, 5-phenyl-3-oxo-pyrazoline) and sodium salicylate produced a significant increase in histamine formation but this was not associated with an increased ulceration index.

2. The ulcerogenic effect of phenylbutazone was aggravated by fasting, adrenalectomy, hypophysectomy, simultaneous adrenalectomy and thyroidectomy, and simultaneous thyroidectomy and vagotomy.

3. Vagotomy reduced the ulcerogenic effect of phenylbutazone in normal rats and adrenalectomized rats, but not in thyroidectomized rats.

4. The effect of phenylbutazone on both the aggressive and defensive factors of the rat gastric mucosa is discussed.

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