

THE INHIBITORY EFFECT ON SOME THIAZIDE DIURETICS AND
ACETAZOLAMIDE ON THE HISTAMINE INDUCED
GASTRIC SECRETORY RESPONSE IN PIGEONS.

By

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Davenport (1) reported the presence of carbonic anhydrase in gastric mucosa and found its distribution to be highly correlated with the distribution of parietal cells. Subsequently, Acetazolamide (Diamox), a potent inhibitor of carbonic anhydrase, was found to inhibit gastric hydrochloric acid secretion in dogs (7); in frogs' isolated gastric mucosa (6); in canine mucosal flaps (12); and in human stomach (3,9).

The thiazide diuretics were synthesised by Novello and Sprague (10) in an attempt to obtain diuretics acting by carbonic anhydrase inhibition in the kidney, and compounds of this series were shown to inhibit carbonic anhydrase *in vitro* (2,12). Chlorothiazide, the parent compound of this series has been shown to inhibit gastric secretion (1, 8). In the present work, the effects of five thiazide diuretics, including chloro-thiazide, have been investigated on gastric secretion.

MATERIALS AND METHODS

The thiazide compounds included in this study were Chlorothiazide, Hydrochlorothiazide, Benzthiazide, Cyclopenthiazide, and Polythiazide. The effect of acetazolamide was also studied for the purpose of comparison with the thiazides. The effect of each of the six drugs was studied on the histamine induced gastric secretion in pigeons by the method developed by one of us (11).

For these experiments a small silver cannula was introduced into the crop of the pigeon aseptically under light ether anaesthesia. Gastric juice was collected by introducing a rubber tube through the silver cannula into the Gizzard (acid secreting part of the pigeon's alimentary canal). The wound healed within 7 to 10 days of the operations after which the experiments were started.

The control gastric secretory response to a standard dose of histamine (0.02 mg of histamine base per 100 gm. body weight), given intramuscularly in the thigh, was determined twice in each pigeon. The volume, free acidity and total acidity of the juice collected for one hour following injection of histamine were determined and the mean of the two control values for each of the above parameter was taken as the standard secretory response for that parameter for a particular pigeon. A gap of several days, as reported earlier (11) was allowed between successive observations.

The thiazide compounds were dissolved in sterile distilled water in the presence of a (pH of the solution varying from 7.4 to 7.6) and injected into the thigh. The volume of the solution injected never exceeded 0.5 ml. The effect of each drug was studied at three doses by administering it 1/2 hr. prior to the injection of the standard dose of histamine.

Free and total acidity of the gastric juice was determined by titrating with freshly prepared 0.05 N sodium hydroxide solution using thymol blue as indicator.

RESULTS

Results have been presented in Table I and II and graphically represented in Figures 1 and 2.

TABLE I

No. of Pigeons	Drug/Control	Dose in mg/100 gm body weight	1 Hour-gastric secretory response to standard histamine		
			Volume in ml.	Free Acid mEq	Total Acid mEq
5	Control Acetazolamide	—	3.73 ± 0.37	238.40 ± 53.3	267.70 ± 56.0
		0.5	2.40 ± 0.10	107.80 ± 27.33	133.40 ± 24.0
		2.0	2.24 ± 0.53	54.00 ± 6.77	68.00 ± 7.0
		4.0	2.06 ± 0.44	31.60 ± 9.66	37.60 ± 6.0
5	Control Chlorothiazide	—	3.87 ± 0.25	241.90 ± 16.85	276.60 ± 47.0
		0.5	2.78 ± 0.17	125.56 ± 31.03	149.40 ± 36.0
		2.0	2.0 ± 0.390	59.20 ± 19.85	70.60 ± 25.0
		4.0	1.64 ± 0.10	38.00 ± 14.45	56.00 ± 17.0
5	Control Hydrochlorothiazide	—	3.61 ± 0.39	244.00 ± 59.20	276.70 ± 56.0
		0.5	2.12 ± 0.21	147.40 ± 15.72	185.60 ± 18.0
		2.0	1.78 ± 0.22	55.60 ± 7.72	71.40 ± 6.0
		4.0	1.28 ± 0.58 (P > 0.01)	40.40 ± 5.94	54.80 ± 8.0
5	Control Benzthiazide	—	3.79 ± 0.51	257.10 ± 57.90	289.00 ± 17.0
		0.5	2.12 ± 0.39	131.64 ± 146.0	162.40 ± 13.0
		2.0	1.62 ± 0.29	56.00 ± 10.61	69.40 ± 11.0
		4.0	1.58 ± 0.31	38.30 ± 3.08	50.60 ± 7.0
5	Control Cyclopenthiiazide	—	3.53 ± 0.34	211.70 ± 9.75	246.30 ± 11.0
		1.0	1.62 ± 0.16	116.60 ± 13.26	131.00 ± 11.0
		2.0	1.64 ± 0.14	93.00 ± 15.07	114.80 ± 11.0
		4.0	1.19 ± 0.17	61.20 ± 14.05	74.20 ± 11.0
5	Control Polythiazide	—	3.01 ± 0.17	138.40 ± 9.77	171.50 ± 11.0
		0.5	2.62 ± 0.26	103.30 ± 7.51	135.40 ± 11.0
		2.0	1.54 ± 0.15	60.00 ± 5.77	81.60 ± 11.0
		4.0	1.52 ± 0.15	43.40 ± 2.67	58.40 ± 11.0

TABLE I shows the volume of gastric juice, the amount of free and total acid secreted within one hour after a standard dose of histamine (Control) and then reduction by acetazolamide and five thiazide diuretics administered 1/2 hour before the histamine injection. Progressive reduction of all the three parameters occurred with increase of doses of the diuretics. All the means were highly significant at 5% level ($P > 0.05$) except where shown in Parenthesis.

A total number of 13 pigeons were used for the present study. Each dose of a drug was studied in five pigeons; this was done with all the six drugs, the pigeons being used inter-changeably for studying more than one drug.

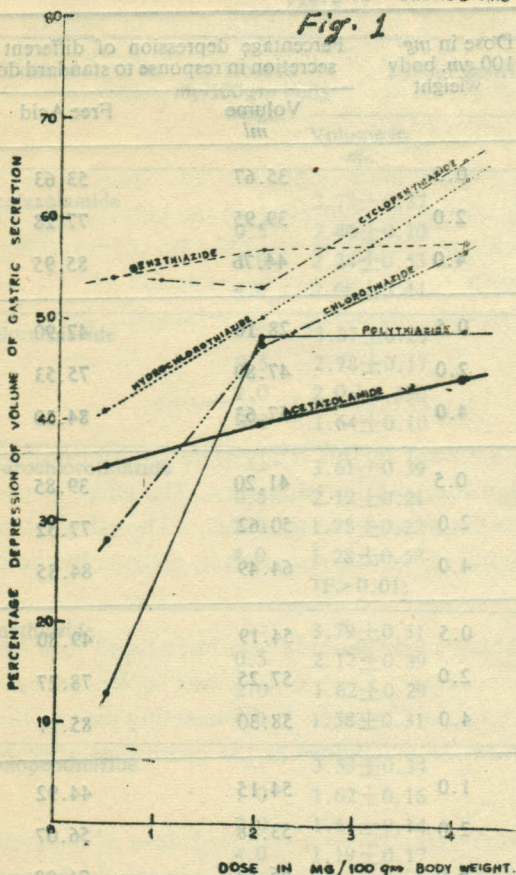
TABLE II

Name of the drug	Dose in mg/ 100 gm. body weight	Percentage depression of different parameters of gastric secretion in response to standard dose of histamine		
		Volume ml	Free Acid	Total Acid
Acetazolamide	0.5	35.67	53.63	53.91
	2.0	39.95	77.28	74.60
	4.0	44.76	85.95	85.95
Chlorothiazide	0.5	28.16	47.90	45.99
	2.0	47.80	75.53	74.47
	4.0	57.63	84.30	79.77
Hydrochlorothiazide	0.5	41.20	39.85	32.89
	2.0	50.62	77.32	74.19
	4.0	64.49	84.85	80.00
Benzthiazide	0.5	54.19	49.80	43.80
	2.0	57.25	78.27	76.01
	4.0	58.30	85.17	82.52
Cyclopenthiiazide	1.0	54.15	44.92	46.81
	2.0	53.48	56.07	53.41
	4.0	66.24	71.08	69.88
Polythiazide	0.5	12.80	25.39	21.04
	2.0	48.72	56.55	52.41
	4.0	49.42	68.65	65.87

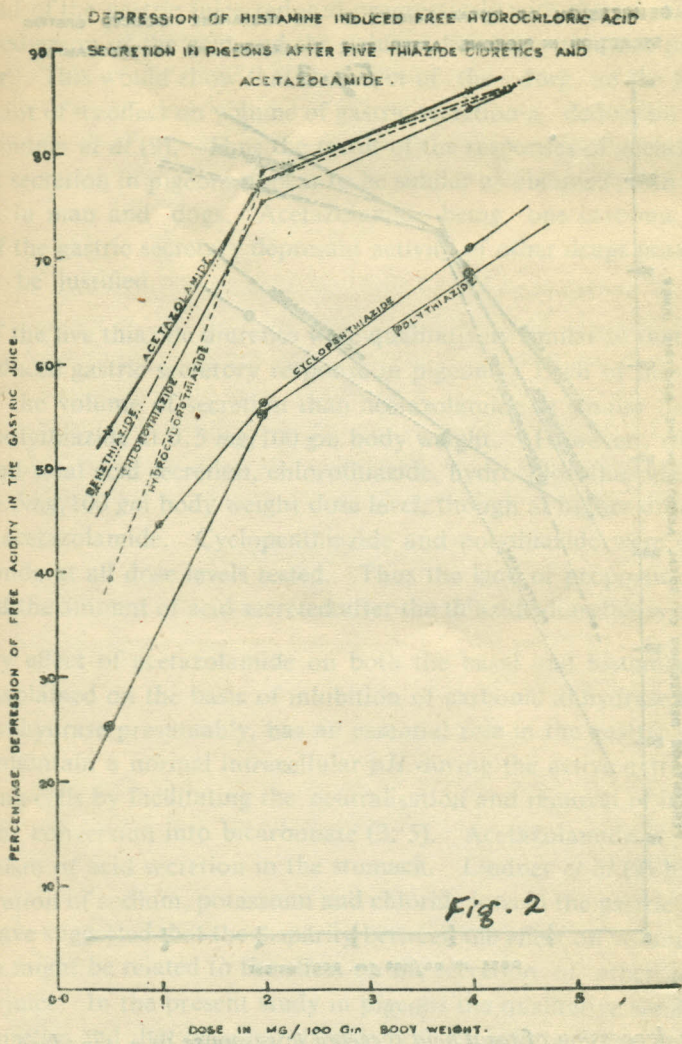
TABLE II shows the percentage reduction of volume, free and total acid content of histamine-induced gastric secretion in Pigeons after increasing doses of Acetazolamide and thiazide diuretics.

DEPRESSION OF VOLUME OF HISTAMINE-INDUCED GASTRIC SECRETION IN PIGEONS AFTER FIVE THIAZIDE DIURETICS AND ACETAZOLAMIDE.

Fig. 1



Showing the depression of histamine-induced gastric secretion in Pigeons after thiazide diuretics and Acetazolamide

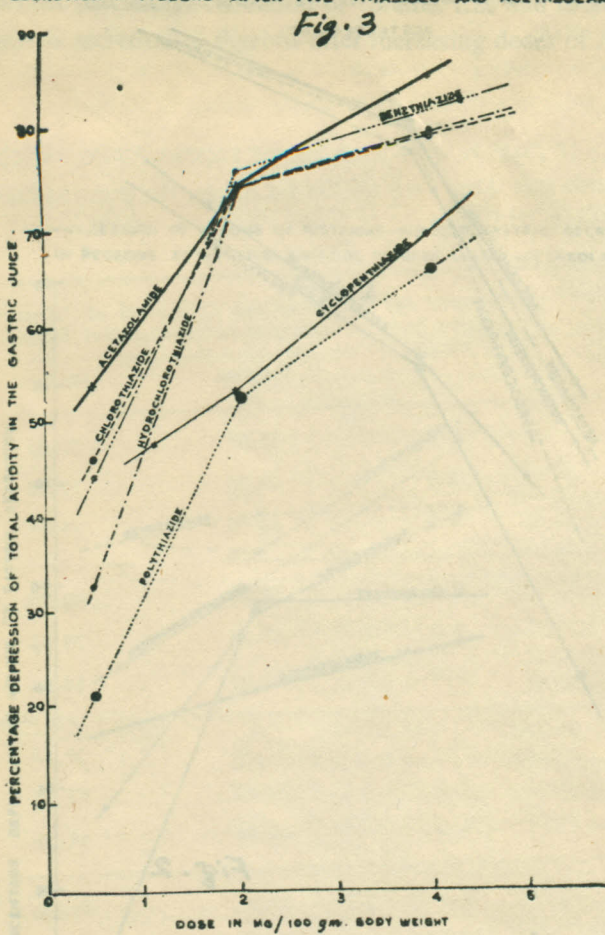


Showing the depression of hitamine-induced Free hydrochoric acid secretion in Pigeons after five thiazide diuretics and Acetazolaminde.

DISCUSSION

The gastric secretory response to a standard dose of histamine (0.02mg. of histamine base/100 gm. body weight) given intramuscularly, at different times to the same pigeon is fairly constant. This has been reported earlier (11), and confirmed in the present study. The diminution occuring in the different parameters of secretory response after pre-treatment with acetazolmide or a member of the thiazide diuretics is highly significant at all dose levels used.

DEPRESSION OF TOTAL ACIDITY OF THE HISTAMINE-INDUCED GASTRIC SECRETION IN PIGEONS AFTER FIVE THIAZIDES AND ACETAZOLAMIDE



Showing the depression of total acid secreted in response to a standard dose of histamine in Pigeons after five thiazide diuretics and Acetazolamide

The effect of acetazolamide observed in this study is qualitatively similar to those observed by the other workers on gastric secretion in dogs and man. Janowitz *et al* (7), administering acetazolamide to dogs with Heidenhain pouch, observed 85% depression of total acid secretion at doses ranging from 20 to 120 mg per kg. body weight. Similar findings were also noted in man by Lindner *et al* (9), who found that the depressant effect of acetazolamide was marked when gastric secretion was augmented by histamine. In this study, acetazolamide doses of 4 mg/100 gm body weight produced nearly 86% depression of both free and total acid secreted within one hour following administration of histamine. The volume; the

HCl and the total acid of the gastric juice reduced progressively with the increase in the dose of acetazolamide; the reduction of the acid and the volume, however, were not parallel or proportionate to each other. This would show that the effect of the drug on the acid secretory mechanism is independent of its effect on volume of gastric secretion a deduction which is in agreement with that of Lindner *et al* (9). Thus the result of the responses of acetazolamide to histamine induced gastric secretion in pigeons appear to be similar as obtained with the same drug on the gastric secretion in man and dogs. Acetazolamide being one carbonic anhydrase inhibitor, comparison of the gastric secretory depressant activity of other drugs possessing similar enzymic activity may be justified.

The effects of the five thiazide diuretics were qualitatively similar to that of acetazolamide on the histamine induced gastric secretory response in pigeons. Each of these drugs produced greater reduction of the volume of secretion than acetazolamide at similar dose levels, except chlorothiazide and polythiazide at 0.5 mg/100 gm body weight. However, with regard to their effects on the free and total acid secretion, chlorothiazide, hydrochlorothiazide and benzthiazide were less potent at 0.5 mg/100 gm body weight dose level, though at higher dose levels they were almost as active as acetazolamide. Cyclopenthiazide and polythiazide were distinctly less potent than acetazolamide at all dose levels tested. Thus the lack of proportion between the volume of secretion and the amount of acid secreted after the thiazide diuretics is obvious.

The inhibitory effect of acetazolamide on both the basal and histamine induced gastric secretion has been explained on the basis of inhibition of carbonic anhydrase in the gastric mucosa (9). Carbonic anhydrase presumably, has an essential role in the gastric secretory process; it possibly helps to maintain a normal intracellular pH during the active extrusion of hydrogen ions from the secreting cells by facilitating the neutralisation and removal of intracellular hydroxyl ions through their conversion into bicarbonate (3, 5). Acetazolamide is thought to affect this primary mechanism of acid secretion in the stomach. Lindner *et al* (9) have also observed a decrease in the secretion of sodium, potassium and chloride ions in the gastric juice of man after acetazolamide and have suggested that the disparity between the effect on volume of secretion and on the acid secretion might be related to the effect on the secretion of other ions (Na^+ and K^+ etc.) into the gastric juice. In the present study in pigeons the qualitative similarity between the effects of thiazide diuretics and that of acetazolamide both on acid secretion and on the volume of gastric secretion suggests that the inhibitory effect of the thiazide diuretics on histamine induced gastric secretory response is possibly due to carbonic anhydrase inhibition *in vivo*. The lack of any anti-histaminic or parasympatholytic activity in these compounds (1) lends additional support to this hypothesis.

On the basis of data presented by Beyer and Baer (2) the *in vitro* carbonic anhydrase inhibitory activity is of highest order in acetazolamide, next in descending order of activity are benzthiazide, polythiazide, chlorothiazide, cyclopenthiazide and hydrochlorothiazide. The order of inhibitory activity on gastric secretion in this study does not conform to their order of car-

bonic anhydrase inhibitory activity *in vitro*. This, however, does not preclude the acceptance of the hypothesis presented above regarding their inhibitory activity on gastric secretion. The *in vivo* enzyme inhibitory activity would depend on several factors like the absorption, distribution characteristics of the drugs and the kinetics of the drug enzyme interaction. However, a definite conclusion cannot be drawn regarding the mode of action of the thiazide diuretics on gastric secretion at present.

SUMMARY

The effects of acetazolamide and five thiazide diuretics, namely, chlorothiazide, hydrochlorothiazide, benzthiazide, cyclopentthiazide and polythiazide, on the histamine induced secretory response have been studied in pigeons. All the drugs diminished the volume of gastric juice and the amount of free and total hydrochloric acid secreted during one hour following the injection of a standard dose of histamine. The reduction in the three parameters of gastric secretion was dependent on the dose of the diuretics. The possibility that this effect of the diuretics might be due to their carbonic anhydrase inhibitory activity *in vivo* has been discussed.

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