PHARMACOLOGICAL STUDY OF ANTIHISTAMINIC PRINCIPLE(S) IN THE *RANA TIGRINA* TISSUES

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Summary: Tissues of the frog, *Rana tigrina* were found to contain antihistaminic principle(s). Skin was found to be its richer and more consistent source than the other tissues. Trichloracetic acid (10% W/V in water) was a suitable solvent for its extraction.

The antihistamic principle was heat-labile but was resistant to trypsin and chymotrypsin; it readily crossed the dialysis membrane.

The frog skin extract inhibited the action of histamine, bradykinin, barium chloride and acetylcholine on the isolated guineaping ileum and rabbit jejunum and that of 5-HT, oxytocin, bradykinin, barium chloride and acetylcholine on the uterus of the oestrogenized rats. The antagonism was short-lived and proportional to the dose of the extract. The extract inhibited the Schultz-Dale phenomenon n the isolated ileum of sensitized guineapigs. In mice it produced sedation and potentiation of the hexobarbitone induced loss of righting reflex.

It did not alter the effects of histamine, acetylocholine, isoprenaline and adrenaline on the blood pressure of the anaesthetized dogs. In small doses the extract did not affect the isolated guineapig heart; larger doses produced depression.

In anaesthetized dogs, the extract considerably reduced the increase in the cutaneous capillary permeability induced by histamine, bradykinin or 5-HT.

It markedly inhibtied the histamine-induced gastric secretion in anaesthetized dogs. In Shay rats, it significantly reduced the volume and the acidity of the gastric contents as also the severity of the ulcer formation.

There is a theoretical possibility that the naturally occuring antihistamines like the frog skin extract may be of some use in peptic ulcer and allergic diseases.

Key Words : *Rana tigrina* naturally occuring antihistaminic principle inhibition of histamine induced gastric acid secretion gastric acidity and gastric ulcers in rats

INTRODUCTION

Tumours of certain plants and, tissues, blood and urine of some mammals contain antihistaminic principles (6,12,15,1,13 & 9). Apparently no work has been reported so far on other animal phyla.

During the estimation of histamine in the tissues of the common Indian frog *Rana tigrina*, Bhide (4) observed that the tissue extracts antagonized the effect of histamine on the isolated guineapig ileum. The present paper describes further work with this material.

MATERIALS AND METHODS

Solvents and extraction: Adult frogs (*Rana tigrina*) of either sex (90—500 *g*) were pithed or were anaesthetized by injecting pentobarbitone sodium (60 *mg/kg*) into the abdominal cavity. Various tissues were cut out, washed in distilled water and dried quickly on filter paper. After weighing, they were transferred to beakers containing individual solvents (5-15 *ml/g* of the tissue) of analytical grade. The tissues were cut into small pieces and left at room temperature (65-102°F) for extraction. When petroleum ether, ether and acetone were used as solvents, the beakers were kept in the refrigerator at about 8°C. After 3-5 days, the supernatant was separated from the tissue residue. The latter was rinsed twice with the solvent and the washings collected and pooled with the supernatant. The pooled extracts were evaporated to dryness under a fan at room temperature.

When the tissues were extracted with solutions of NaOH or Na_2CO_3 or HCl, the evaporation was omitted but the pH of the extract was brought to 7 by using narrow range pH indicator paper prior to using the extract for biological assay.

When the tissues were extracted with trichloracetic acid, the supernatant was shaken 400 times with 4 volumes of ether on 4 successive occasions, to remove the trichloracetic acid (3). The remaining aqueous extract was then briefly and gently warmed to remove traces of ether. Extracts with trichloracetic acid were also made with the skin of 3 toads(*Bufo melanostictus*), 1 house lizard (*Hemidactylus flaviviridis*), 3 albino rats and 2 guineapigs.

The extracts of tissues were diluted with distilled water such that 1 *ml* would correspond to 200 *mg* weight of fresh tissue. The extracts of tissues obtained from at least 2-5 animals were assayed for antihistaminic activity on the isolated guineapig ileum. The results of these experiments indicated that trichloracetic acid solution (3% or 10% W/V in water) was the most efficient solvent. *The frog skin extract thus prepared is hereafter referred to as "the extract" and, its dose refers to the weight of fresh skin.* For convenience, about 10-30 *g* of frogs' skin was extracted with trichloracetic acid, processed with ether and then stored at 8°C in stoppered glass bottles. Under these conditions, the antihistaminic potency of the extract did not decline during 10 weeks.

Pharmacological experiments: The effect of the extract was studied on isolated guineapig ileum, isolated uterus of rat in oestrus and isolated rabbit intestine according to methods detailed elsewhere (17). The capacity of the isolated tissue bath was 9 *ml* and the magnification of the lever was 7 to 10.

Schultz-Dale reaction: Adult guineapigs of either sex were injected ip, on 2 successive days, 5 *ml* of fresh egg-white solution (25% V/V in water); or, in some animals, 2 *ml* horse serum was twice injected ip at the interval of 3 hr. After 21-30 days, they were killed and pieces of ileum were mounted in the isolated organ bath as described above. The response of individual pieces to 0.1 *ml* of fresh egg-white solution or horse serum with or without different doses of the extract was recorded.

Isolated guineapig heart : Langendorff's method (7) was followed for recording the amplitude of contraction of the guineapig heart.

Blood pressure of the dog: Dogs of either sex (7-15 kg) were annesthetized with pentobarbitone sodium (35 *mg/kg ip*). Carotid artery blood pressure was recorded on smoked paper with mercury manometer; drugs were injected through the cannulated femoral vein.

Hexobarbitone-induced loss of righting reflex in mice: The extract (0.1-1.0 g/kg) or comparable volume of distilled water was injected ip into albino mice (25-45 *g*) of either sex. This was followed, 20 min. later, by 50 *mg/kg* hexobarbitone sodium injected by the same route. The duration of loss of righting reflex by individual animals was noted.

Effects on capillary permeability: Light brown dogs of either sex were anaesthetized with pentobarbitone sodium (35 *mg/kg ip*). The hair on the abdomen was carefully clipped, without causing injury to the skin. Evans blue dissolved in distilled (water 50 *mg* in 10 *ml*) was injected intravenously. Within 10 min, suitable solutions of the agonists in normal saline were injected intradermally at various sites on the abdomen and, in addition, solutions of the agonists mixed with various concentrations of the extract or mepyramine maleate. Normal saline, the extract and mepyramine were separately injected intradermally as controls. The volume of the fluid injected at any single site was 0.1 *ml*. One hr. later, the diameter of individual areas of blue discolouration at sites of intradermal injections was measured.

Histamine-induced gastric secretion in dogs: Dogs of either sex (5-15 *kg*) were used. After 20 hr starvation (water allowed), they were anaesthetized with pentobarbitone sodium (35 *mg/kg ip*). After a midline abdominal incision, the pylorus was exposed and ligated; the stomach was opened by a small cut on its ventral surface about 4 *cm* proximal to the pylorus. A short glass cannula (7 mm diameter) was left in this opening to keep it patent and the stomach contents were aspirated by a soft rubber catheter. To ensure complete removal of food particles at the beginning of the experiment, the stomach was washed repeatedly with 10-15 *ml* distilled water (37°C).

Histamine was injected sc (13) and the gastric contents were completely aspirated at 15 min intervals; the gastric secretary stimulation by histamine usually subsided within 10 min (Fig. 5). The 15 min values of volume and acidity obtained over this period were pooled and considered as the individual response to histamine injection (Table III). In the control study, histamine injections were repeated after the gastric secretary rate returned to the basal level. In the other groups of animals, after obtaining the control response to histamine, the extract (0.3-2.5 *g/kg*) was injected intravenously and the response to subsequent injections of histamine was studied. Individual dogs received only one dose of the extract.

Gastric juice sample were titrated with 0.1 N NaOH using Topfer's reagent and phenolphthalein as Indicators to estimate the free and total acid respectively.

Shay method (13): Adult albino rats of either sex were deprived of food (but not of water) for 48 hr. Under ether anaesthesia, the pylorus was ligated, the extract or distilled water was injected into the duodenum and the abdominal wall was closed. Following denial of food and water for the next 18 hr, the animals were killed, the stomach was carefully taken out and, afetr measuring the volume, the acidity of the gastric contents was determined as described above. The

ulcers on the stomach mucosa were graded as follows and the ulcer index calculated by the meth of Pauls *et al.* (14).

Types of ulcers	*Grades*
Small pinpoint ulcers (according to size and number of ulcers persent)	± to ++
Large, well demarcated ulcers	++± to +++
Haemorrhagic and perforated ulcers	+++± to +++

RESULTS

Solvents: Of the various solvents used, water petroleum ether, benzene, chloroform and diethyl ether failed to extract the antihistaminic principle from the frog skin as did aqueou solutions of ethanol (20, 50 and 96% V/V), methanol (20, 50 and 96% V/V), acetone (20 and 50% V/V), HCl (0.5, 3.0 and 10.0% W/V), $Na_2 Co_3$ (0.5 and 3.0% W/V) and NaOH (0.5 and 3.0% W/V) Extracts with acetone (pure) and 1% W/V trichloracetic acid in water had some activity. However with any individual tissue sample the highest yield was obtained by 3% and 10% W/V trichloracetic acid in water.

Distribution of the antihistaminic principle in various tissues: The skin of the frog was the richer and more consistent source of antihistaminic principle than the other tissues studied (Tab I). The antihistaminic potency of the extracts of the skin pieces obtained from the upper jaw the back, the abdomen and dorsal and ventral surfaces of the hind limbs of the individual frogs wa almost identical. Extracts prepared between February and November were invariably acti thus while the extract of 20-100 *mg* of the skin obtained during this time of the year could abolis the contractile response of isolated guinea-pig ileum to 1 μg histamine, extracts of as much a 0.5 *g* skin of the frogs killed in December were found to be devoid of antihistaminic activit Also, there was variations in the potency of the samples obtained from the individual frogs. Th sex and weight of the animal were apparently unrelated to this variation. When the frog ski pieces were repeatedly extracted with aliquots of 10% trichloracetic acid, about half of the antih taminic activity was recovered in the first 24 hr. and, the remaining in the next 48 hr. Prolonge extraction for beyond 48 hr did not yield any additional antihistaminic activity.

The sample of the extract from 1 *g* skin yielded about 56-60 *mg* dry residue and the pH of the extract was about 6.5. Estimated by flame photometer, extracts from 1 g skin sample contained 0.4—0.7 *mg* potassium (n=3).

Trichloracetic acid extract of the skin of toad and house lizard (but not of rat and guineapig showed antihistaminic activity. Extracts of about 250 *mg* skin completely blocked the action of 0.1—0.3 μg histamine in 9 *ml* bath volume.

Stability of the extract: The frog skin extract stored at 32°C for 8 days did not lose it antihistaminic activity; however, when kept for 12 days, it lost over 50% activity. Addition of concentrated Hcl to make the pH of the extract 1 or of NaOH to make the pH 11 did not inhibit the rate of inactivation at 32°C. The extract, however, lost considerably its antihistaminic acti

vity when kept at these two extreme pH for 24 hr. Addition of ascorbic acid (10 *μg/ml*) as an antioxident or of a layer of petroleum ether on top of the extract (which cuts it from the atmospheric oxygen) for 7 days could not improve its stability, indicating that oxidative reaction may not be the important cause of inactivation of the extract at room temperature. Storage of the extract at low temperature (8°C) for as long as 10 weeks did not alter the antihistaminic potency, while even one hour's exposure of it to 100°C caused a complete loss of the activity.

TABLE I: Antihistaminic activity in tissues of *Rana tigrina* killed between August and November. Tissues were extracted with 10% trichloracetic acid.

Tissues	*Number of animals used*	*Number of extracts which had antihistaminic activity*	*Approximate antihistaminic potency of the tissue extracts*
Skin	19	19	++++
Thigh muscles	14	6	+++
Gastrocnemius muscle	14	6	+++
Rectus abdominis muscle	13	9	++
Lungs	13	6	+
Heart	13	6	+
Kidneys	13	8	++
Tongue	7	2	++
Stomach	13	4	±
Liver	12	9	+
Intestine (small and large)	13	5	+++

The 4 grades of antihistaminic effect (++++,+++,++ and+) indicate 50% inhibition of the histamine response (0.3) *μg* in 9 *ml* bath volume) of the guineapig ileum by the extracts of tisues weighing 10-125 *mg*, 126-250 *mg*, 251-375 *mg* and 376-500 *mg* respectively. When 500 *mg* extract produced about 25% inhibit on it was taken as ±.

The antihistaminic activity was not lost when the extract was incubated with trypsin (0.35 mg/ml) or chymotrypsin (0.2 *mg/ml*) at 37°C at the pH 8 for 4 hr. A similar treatment of bradykinin, a polypeptide abolished its activity as tested on the isolated guineapig ileum.

Attempted purification: When the extract was added to 3 volumes of cold (8° C) acetone, there was a dense precipitate. This was dried and weighed; the acetone-water supernatant was also evaporated. Although the residue from the supernatant was only about half the weight of the precipitate, it had 4-12 times more antihistaminic acticity on weight basis.

After 18 hr dialysis in distilled water, no antihistaminic activity could be detected in the dialyzed extract.

Pharmacolgical study:

Isolated guineapig ileum: The extract reduced or abolished the contraction of the guineapig ileum induced by histamine, bradykinin, barium chloride and acetylcholine, (n=10). In a 9 *ml* bath, usually the frog skin extract (30 *mg*) reduced the response of the tissue to histamine

acid phosphate (1 μg), bradykinin (0.2 μg) and barium chloride (1 *mg*) by about 100, 30 and 50% respectively; as much as 300 *mg* was unable to inhibit the response to acetylcholine (1 μg). The antagonism was short-lived and increased with larger doses of the extract (Fig. 1) and could be overcome by increasing the dose of the agonists. When mixed, *in vitro* with histamine and left for 15 min, the extract produced the same total effect as when the two were separately added to the organ bath. In doses of 0.1-2.0 μg (per 9 *ml* bath volume) adrenaline and isoprenaline blocked the action of 1 μg of histamine.

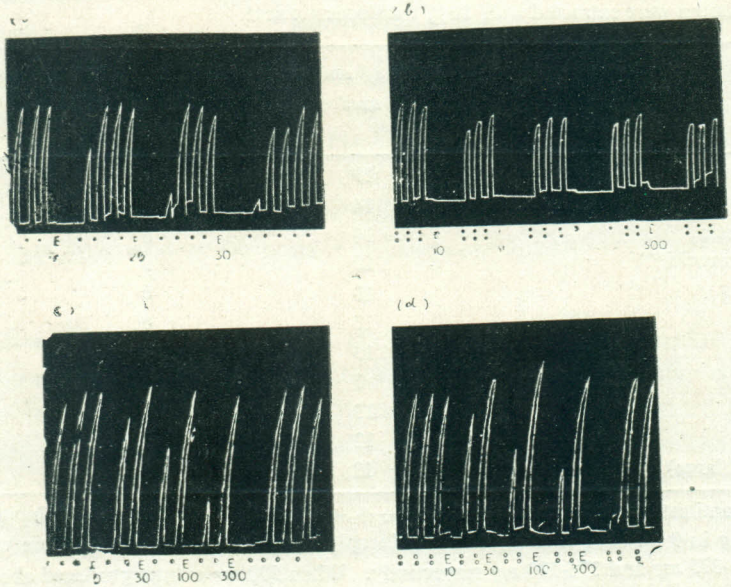

Fig. 1; Isolated guineapig ileum. Dose-response cycle 3 min. Doses per 9 *ml* bath volume. Frog skin extract (E, Hindu figures represent the weight of the fresh skin in *mg*) was added 30 sec before (a) histamine (• = 1.0 μg), (b) acetylocholine (: = 1.0 μg), (c) bradykinin (o = 0.2 μg) or (d) barium chloride (: = 1.0 *mg*).

Oestrogenized rat uterus: The extract reduced the contraction of the rat uterus induced by 5-HT , acetylcholine, bradykinin, oxytocin and brarium chloride (n=5). Again, the antagonism was short-lived and greater with larger doses (Fig. 2, 3).

Isolated rabbit jejunum: The extract produced dose-related relaxation of the rabbit intestine. It also antagonized the action of histamine, bradykinin, barium chloride and acetylcholine (n=5).

With the 3 isolated tissues mentioned above, it was found that, for blocking the action of acetylcholine, about 3-4 times more extract was required than that for blocking the action of other agonists.

Schultz-Dale reaction: Contraction of the isolated intestine of the senstized guineapigs (n=3) induced by the antigen could be completely suppressed by 0.1-0.3 g of the extract in 9 *ml* bath volume (Fig. 4).

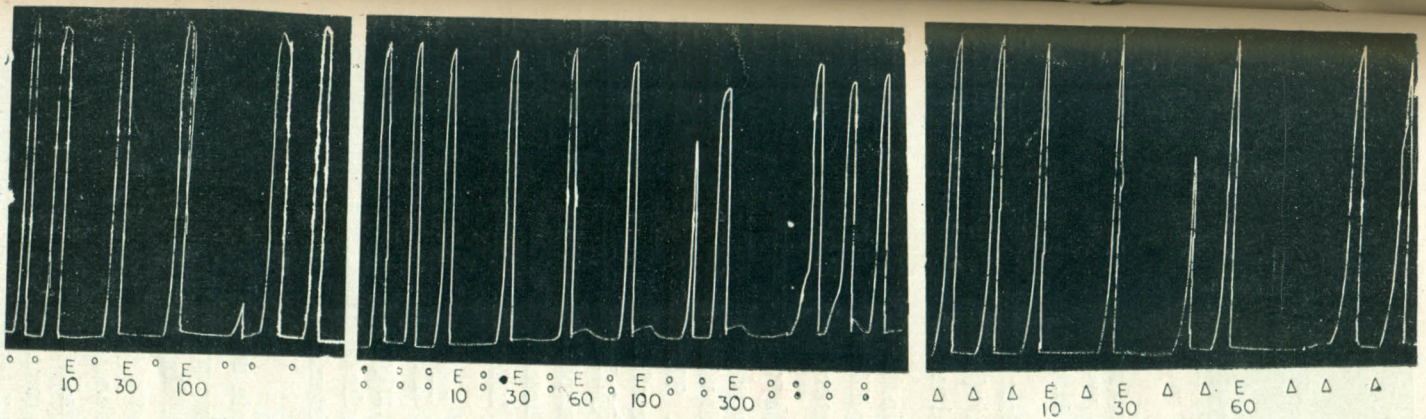

Fig. 2: Isolated uterus of the oestrogenized rat. Dose response cycle 4 min. Doses per 9 *ml* bath volume. Frog skin extract (E, Hindu figures represent the weight of the fresh skin in *mg*) was added 30 sec before 5-HT (o = 0.01 μg), acetylcholine (: = 5.0 μg) or bradykinin (△ = 0. 1 μg).

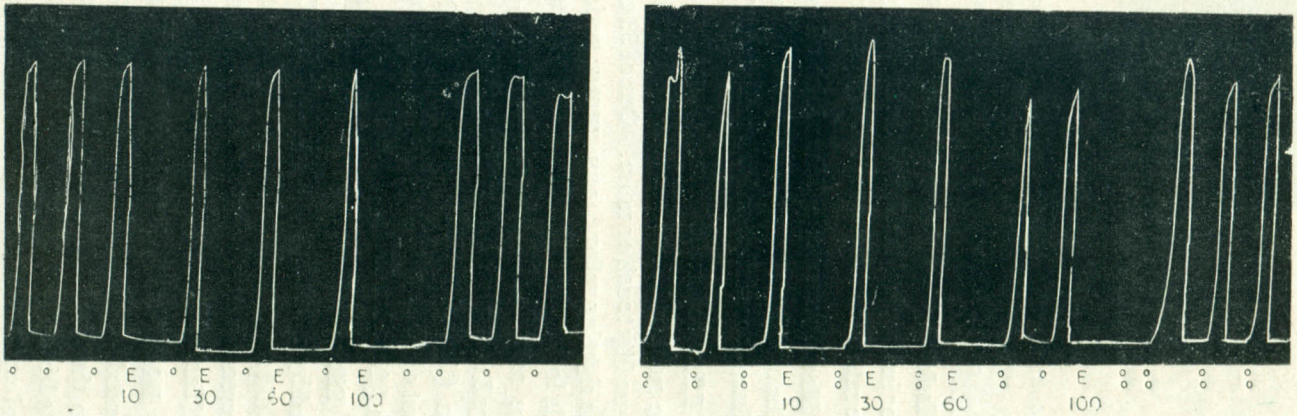

Fig. 3: Isolated uterus of the oestrogenized rat. Dose-response cycle 4 min. Doses 9 *ml* bath volume. Frog skin extract (E, Hindu figures represent the weight of the fresh skin) was added 30 sec. before oxytocin (o = 0.05 I.U.) or barium chloride (: = 1.0 *mg*).

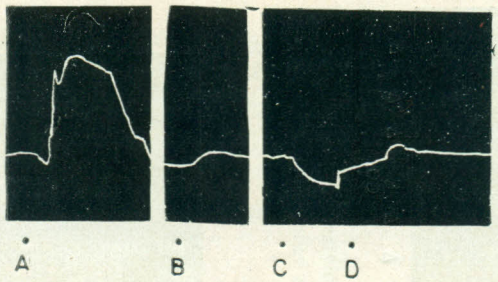

Fig. 4: Schultz-Dale reaction of 3 pieces of ileum of a sensitized guineapig. A - 0.1 *ml* horse serum (antige
B - 100 *mg* frog skin extract and 0.1 *ml* horse serum added in quick succession; C-200 *mg* frog sk
extract followed after 30 sec. by 0.1 *ml* horse serum, D.

Isolated guineapig heart perfusion: Upto 50 *mg* dose, the extract did not alter the chrono
tropic or inotropic function of the isolated guineapig heart (n=6). High doses reduced the force
and the rate of contraction; 300 *mg* dose produced rapid cardiac arrest in diastole.

Blood pressure: When injected intravenously in 0.3-2.0 *g/kg* doses, the extract did not
affect the blood pressure of the anaesthetized dogs; nor did it alter the actions of histamine, ace
tylcholine, isoprenaline and adrenaline on the blood pressure (n=8).

Effect on hexobarbitone-induced loss of righting reflex: On receiving the extract, the mice
became quiet and there was narrowing or closure of the palpebral fissure; however, they could
be aroused by touch or noise. Out of 33 control animals which received only hexobarbitone, 1
lost the righting reflex for an average period of 13 min. The index of loss of righting reflex (%
animals losing the reflex X the average duration in min of the loss of reflex) was 507. Out o
15 mice receiving 0.1 *g/kg* of the extract, 12 lost the righting reflex after hexobarbitone for th
average 8 min period (the index - 640); with 1 *g/kg*, 11 out of 15 mice lost the reflex for the averag
24 min (the index-1752). In another experiment conducted in this laboatory (17) it was foun
that out of 15 mice receiving 5 *mg/kg* chlorpromazine, 12 lost the righting reflex for the averag
22 min (the index-1804).

Effect on the cutaneous capillary permeability: The increase in capillary permeabilit
induced by histamine, bradykinin and 5-HT could be clearly reduced by the extract. The extract
alone did not significantly increase the permeability. In the dose used here, mepyramine blocke
the increase in capillary permeability induced by the histamine but not that by bradykinin o
5-HT (Table II).

Inhibition of histamine-induced gastric secretion in dogs by the extract: The animal
responded well to 15-30 $\mu g/kg$ histamine and in all these experiments, the changes in the volume
and in the free and total acidity were nearly parallel. In individual animals, the response to
repeated injections of a particular dose of histamine was generally uniform (Fig 5-i). In the
control experiments where 14 dogs received histamine alone, compared to the 14 initial response
to histamine, only 7 out of 27 subsequent responses indicated 4-38% lower acidity and, only
2 of these 7 indiated 28-38% reduction. The animals remained sensitive to histamine for about
6-10 hr which permitted study of the response to 4-7 injections of histamine.

In 8 dogs, when the extract was given intavenously, it invariably and impressively reduced the response to histamine. With the higher doses of the extract, the response to histamine was completely abolished during the remaining hours of the experiments; at this stage, doses of histamine 2-10 times higher than the initial ones failed to produce significant response (Fig. 5-ii). Thus, compared to the 8 initial responses to histamine, all the subsequent 29 responses obtained after the injections of the active extract indicated 15-100% decrease in acidity and 25 out of these 29 indicated 50-100% inhibition.

A sample of the frog skin extract which was only about 1/20th as potent antihistamine (when assayed on guineapig ileum) as those used above was also tested in 3 dogs as a negative control. It did not produce discernible inhibition of histamine-induced gastric secretion.

TABLE II : Effect of the frog skin extract and mepyramine on the increase in cutaneous capillary permeability induced by histamine, bradykinin and 5-HT in anaesthetized dogs.

Agonist (dose)	*Weight of the frog skin present in the extract which was mixed with agonists*	*Dose of mepyramine maleate*	*Increase in the capillary permeability (number of experiments)*
Histamine acid phosphate (1.0 μg)	—	—	++++ (9)
	5 *mg*	—	+++± (3)
	10 *mg*	—	+++ (3)
	20 *mg*	—	++ (3)
	40 *mg*	—	± (3)
	—	5 μg	++ (2)
	—	10 μg	+ (1)
	—	15 μg	± (1)
Bradykinin (0.2 μg)	—	—	++++ (9)
	5 *mg*	—	+++ (2)
	10 *mg*	—	++ (3)
	20 *mg*	—	+ (3)
	40 *mg*	—	± (3)
	—	5 μg	++++ (1)
	—	15 μg	++++ (1)
5-HT creatinine sulphate (0.1 μg)	—	—	+++ + (4)
	5 *mg*	—	++ (2)
	10 *mg*	—	+ (2)
	20 *mg*	—	± (2)
	40 *mg*	—	Nil (2)
	—	5 μg	+++ + (1)
	—	15 μg	+++ + (1)
Nil	20 *mg*	—	Nil (2)
	40 *mg*	—	± (6)

The capillary permeability increase was assessed by measuring the diameter of the blue-stained area. Grades ±, +, + +, + + +, + + + + indicate 6-8 *mm*, 8-10 *mm*, 10-12 *mm*, 12-14 *mm*, and 14-16 *mm* diameter respectively.

Shay method: The extract-treated rats showed statistically significant reduction in the ulcer grade, ulcer index and volume and acidity of the gastric contents (Table III). Whereas all the control animals developed ulcers of the stomach, many receiving the extract did not. At 1.5 *g/kg* dose of the extract, the response was better than at 0.5 *g/kg* dose; increasing the dose to 5 *g/kg* did not produce better results.

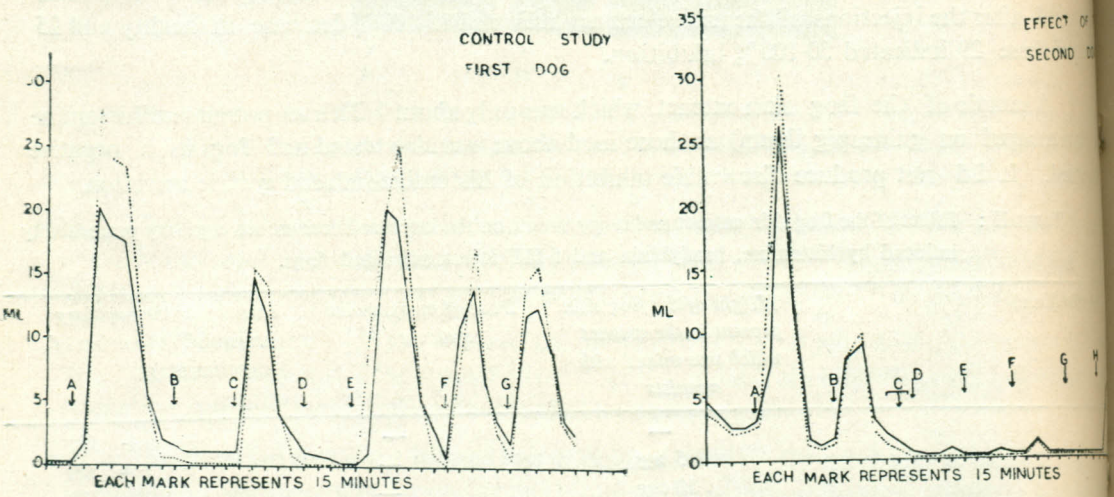

Fig. 5 (i)&(ii): Gastric secretary response of 2 anaesthetized dogs receiving histamine subcutaneously. Cont represents the volume of gastric contents collected and dotted one, the total acidity. (i) control study; 27 μg/kg of histamine at A and E; 13.5 μg/kg of histamine at C, F and G of distilled water (subcutaneously injected) at B and D. (ii) Second dog: 15 μg/kg o injected at A, F and G; 10 μg/kg of histamine at B, D and E; 30 μg/kg of histamine at and 1.3 *g/kg* of frog skin extract at C.

TABLE III: Effect of the frog skin extract on the gastric acid secretion and on the ulcer formation in Shay rats. The extract was injected into the duodenum. S.D. = standard deviation; P calculated by t-test; NS = not significant when $P > 0.05$. Ulcer index = average ulcer grade X percent animals showing ulcers.

Group (number of rats)	*Dose of the extract (g/kg)*	*Average volume of gastric juice ml ± S.D. (p)*	*Average gastric free acid in terms of 0.1 N NaOH ml ± S.D.*	*Average gastric total acid in terms of 0.1 N NaOH ml ± S.D.*	*Average grade of ulcer ± S.D. (p)*	*Ulcer index*
Control (24)	Nil (1 *ml* distilled water)	4.8 ± 0.53	1.03 ± 0.28	2.42 ± 0.46	2.05 ± 0.24	206
The extract (11)	0.5	3.34 ± 0.8 (NS)	0.68 ± 0.34	1.82 ± 0.55 (NS)	1.22 ± 0.32 (<0.05)	111
The extract (10)	1.5	1.93 ± 0.54 (<0.01)	0.18 ± 0.1	0.77 ± 0.35 (<0.01)	0.5 ± 0.15 (<0.001)	30
The extract (15)	5.0	1.93 0.75 (<0.01)	0.71 ± 0.44	1.08 ± 0.65 (<0.01)	0.69 ± 0.02 (<0.01)	44

DISCUSSION

In the present work, the term "antihistaminic" is used for convenience and because, as in the previous reports (reviewed by Kovacs *et al.*, 9), antagonism to histamine was the first to be detected and studied. The term does not imply the highly specific antihistamine action which characterizes synthetic antihistamines.

Because the antihistaminic priciple is often found in many other tissues of the frog (Table I), it cannot be considered to be peculiar to its skin alone. Antihistaminic activity was detected in the skin of the toad and house lizard; however, no further work was conducted in these species.

The chemical structure of several naturally occurring antihistaminics studied so far is not known (9), and, in the present work, the experiments have been conducted with the crude or partially purified extracts. Although the data presented here are insufficient for ascertaining the chemical structure of the antihistaminc principle in the frog tissues, some preliminary work has been done. Since it is inactivated on boiling, it is unlikely to be an inorganic substance. Since 10% trichloracetic acid (which denatures and precipitates proteins) could extract it out from the tissues, it is not likely to be a large protein. Unlike bradykinin, the extract was not inactivated by trypsin or chymotrypsin; therefore, it is not likely to have comparable polypeptide structure. Its rapid passage across the cellophane dialysis membrane indicates comparatively small molecular size. Comparative rates of elution through the sephadex G-50 column indicate that the molecular weight of the active material is probably less then 1200 (Authors' unpublished observations).

Catecholamines like adrenaline and isoprenaline do inhibit the spasmogenic action of histamine and other agonists on the rsolated smooth muscle preparations. On the isolated guinea-pig ileum, 0.1-2.0/μg of adrenaline or isopanaline had inhibitory effect comparable to that by 20-70 *mg* of the frog skin extract. On the other hand, in doses which produced marked inhibition of histamine-induced gastric secretion (0.3-2.5 *g/kg*) the extract, unlike adreanline or isoprenaline did not alter the blood pressure of the anaesthetized dogs. Also, on the isolated guineapig heart, the extract had only inhibitory effects. These findings, render it unlikely that the antihistaminic principle in the frog skin extract is adrenaline or isoprenaline-like.

In terms of fresh tissue weight, the frog skin extract appeared to possess greater antihistaminic activity on the isolated guineapig ileum than those of normal and malignant human tissues (12, 15) and bovine thymus (9).

On the isolated tissue preparations, larger doses of the extract were required for blocking the action of acetylcholine than those for blocking the action of other agonists. This finding is in agreement with those of Kovacs and Melville (10) and Kovacs *et al.* (12) on other naturally occurring antihistamines.

Several synthetic antihistamines induce sedation and potentiate the action of barbiturates in man and animals. The antihistamine from the crown gall tumours induces strong sedation in the guineapigs (6) and that in the pregnant mare urine produces hypnosis, hypothermia and hexobarbitone potentiation (18). The frog skin extract also induced sedation in mice and potentiated the action of hexobarbitone.

The extract, like some other naturally occurring antihistamines (11) inhibited the increase in capillary permeability induced by histamine, bradykinin and 5-HT and it was nearly equally effective against the 3 agonists. In doses which blocked the histamine-induced increase in capillary permeability, mepyramine, a synthetic anti-histamine had no inhibitory action against bradykinin and 5-HT (Table-II).

Synthetic antihistamines do not block the action of histamine on the gastric acid secreting glands. Indeed this anomalous situation has led to the concept that histamine receptors on these glands might be different from those on the intestinal smooth muscles (2). It is, therefore, interesting to note that the naturally occurring antihistamines from human and horse urine (13) and from the frog skin (present report) could inhibit histamine-induced gastric acid secretion in the mammals. Also, all of them could significantly reduce ulcers and, volume and acidity of the gastric juice in Shay rat preparation (13 and present report). Therefore, it would be worth an attempt to see if the naturally occurring antihistamines are of value in clinical peptic ulcers.

The ability of the naturally occurring antihistamines to inhibit the smooth muscle contractions induced by so many agonists and antigen has been described as "the blanket activity" (9) and it is fully shared by the frog skin extract. So far, they have not been used in allergic conditions like bronchial asthema although, on theoretical ground, they might prove useful. In this connection, it is curious to note that the Thakur tribe of the hilly area near Bombay eat, for the treatment of bronchial asthma, the powder of frogs; for this purpose, large-size frogs are killed in the month of May, eviscerated, smeared with turmeric powder, dried in the sun and then powdered (Chapekar, 8).

The contribution of naturally occurring antihistamines to the survival of the organism is not known. However, in view of their widespread occurrence, it is suggested that they may act, *in vivo*, as antigonists of histamine, bradykinin and 5-HT a possibillity entertained by Kovacs *et al.* (12). Consistent with this suggestion is the fact that the frog (*Rana tigrina*) with higher tissue antihistamine activity than the mammals, is very insensitive to histamine (5, 16) and to bradykinin and 5-HT (4). (4). However, more information is required for verification of this role suggested for the naturally occurring antihistamines.

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