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HISTAMINE PROTECTION PRODUCED BY PLANT TUMOUR EXTRACTS. THE ACTIVE PRINCIPLE OF TOMATO PLANTS INFECTED WITH GROWN-GALL

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Guinea-pigs were protected against the lethal effects of a histamine aerosol by intraperitoneal injection of stable extracts of normal tomato plants and of tomato plants infected with crown-gall tumours. The protection was short-lasting. No difference was observed between the activities of extracts of normal and of infected plants. The active principle of the extracts was isolated, and identified as the steroid alkaloid glycoside, tomatine.

The antihistamine activity of plant tumour extracts was first demonstrated by Kovacs & Szabadi (1950) and Kovacs, Kovacs, Szabadi & Varsanyi (1952) who showed that intraperitoneal injection of extracts prepared from oak galls protected guinea-pigs against the lethal effects of a histamine aerosol. This observation was confirmed and extended to extracts obtained from tomato plants infected with crown-gall tumours (Broome, Callow, Feldberg & Kovacs, 1962). The active constituent was present in chloroform-extracts of the ethanol-soluble portion of infected tomato plants. Uninfected plants of another strain showed some activity, but plants of the same type and age as the infected ones were not then available for comparison.

This paper describes further studies with tomato plants, both normal and infected with crown-gall tumours, grown under the same conditions. An improved method for preparation of active material was developed. An aqueous ethanolic extract from the plants was evaporated to small volume and the resulting aqueous solution submitted to a modified countercurrent distribution with chloroform as the organic phase. Antihistamine activity was detected in the aqueous phase, and, after chromatography of this on Sephadex, was associated with an alkaloid-containing fraction. The active principle was isolated by chromatography on alumina and identified as the steroid alkaloid glycoside, tomatine.

The tomatine molecule (Fig. 1) consists of a branched tetrasaccharide chain, containing two molecules of D-glucose, and one each of D-galactose and D-xylose (Kuhn, Löw & Trischmann, 1957), attached to a steroidal nucleus, tomatidine (5α,22β,25-L-spirosolan-3β-ol). The structure of tomatidine was demonstrated by partial synthesis from neotigogenin (Uhle & Moore, 1954). The absolute configuration of the nitrogen-containing ring F has only recently been confirmed (Boll

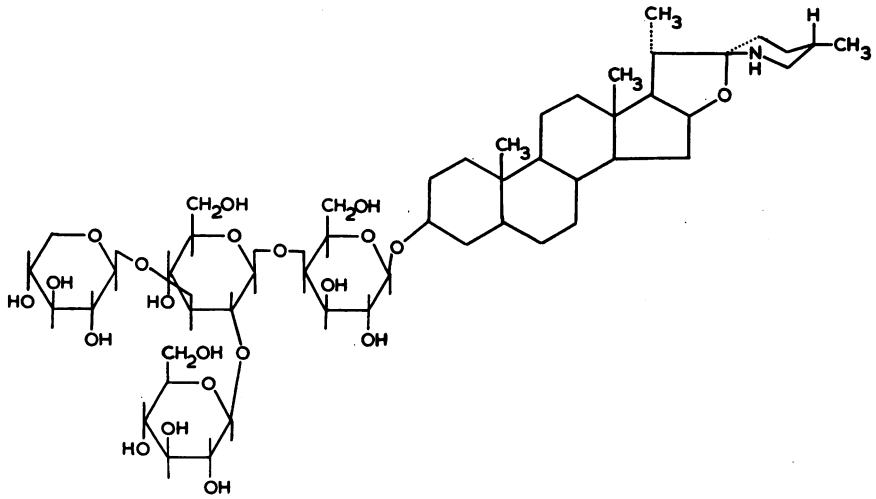

Fig. 1. The structure of tomatine.

& Sjöberg, 1963) and a formal total synthesis of tomatidine published (Schreiber & Adam, 1963).

METHODS

General methods

Exposure of guinea-pigs to histamine aerosol. The method has been described previously (Broome *et al.*, 1962), but a more concentrated histamine aerosol was used in the present series of investigations. Male guinea-pigs, most weighing between 200 and 250 g, were injected intraperitoneally with extracts made up in saline, 0.9% (w/v), and their controls injected with an equal volume of saline. Pure steroid alkaloids were dissolved in saline containing acetic acid (about 1%, v/v). Control animals appeared to be unaffected by this acid solution. After 4 to 6 hr from injection, one control and one or two test animals were simultaneously exposed in the same chamber to an aerosol containing histamine acid phosphate (1%, w/v) and the time breathing stopped in each was noted. The maximum period of exposure was 20 min. Four animals were tested with each extract to allow for variation in sensitivity to the aerosol.

Paper chromatography. Chromatograms were developed on Whatman No. 1 paper by the descending technique. The solvent systems were: (A) ethyl acetate—acetic acid—water (11:2:1.85, by volume) (Briggs, Cambie & Hoare, 1961); and (B) the upper layer of ethyl acetate—pyridine—water (3:1:3, by volume) (Schreiber, 1958).

Thin-layer chromatography. This was performed on glass plates coated with silica gel G (E. Merck AG, Darmstadt). The thickness of the film was about 250 μ and the solvent front travelled about 14 cm in 2 hr. The solvent systems were: (B) as for paper chromatography; and (C) the lower layer of chloroform—ethanol—ammonia solution (2:2:1, by volume) (Boll, 1962). The ammonia solution was prepared by diluting concentrated ammonia (specific gravity, 0.890; 1 volume) with water (99 volumes).

Location and identification. Alkaloids were routinely located by spraying chromatograms with the Dragendorff reagent of Thies & Reuther (1954). This reagent also locates choline which gives a red spot in contrast to the more orange spots given by alkaloids. With solvent system B it was necessary to remove pyridine completely from the chromatogram by drying at 105° C before spraying. On a few occasions, chromatograms were sprayed with antimony trichloride in chloroform (25%, w/v) and heated at 110° C for 5 min to detect 3-hydroxy-

steroids. Compounds were identified by means of markers run on the same chromatogram, and by mixed spots.

Preparation of plant extracts

Plants. Crown-gall tumours were produced by artificial infection with *Agrobacterium tumefaciens*. Control and infected tomato plants (variety Sutton's " Best of All ") were grown under identical conditions at the Glasshouse Crops Research Institute, Littlehampton, Sussex. Plants were received intact and were separated into leaves, stems, roots and green fruit. Control and infected plants were treated separately by identical procedures.

Preliminary extraction. The improved method of preparation was based on preliminary experiments carried out with healthy and with infected stem tissue. Active extracts could be obtained from both types of plant material by direct extraction with ethanol without recourse to anaerobic conditions.

Leaves were packed into polyethylene bins, covered with aqueous ethanol (95%, v/v) and left for at least 1 week. The extract was separated in a basket centrifuge, and the partially pulped leaves re-extracted twice in the same way. The three ethanolic solutions were combined, and ethanol removed in a cyclone evaporator until a largely aqueous solution remained. This was extracted twice with an equal volume of light petroleum (boiling point 40 to 60° C), and concentrated to a deep brown solution. The light petroleum extract was discarded.

The other plant tissues were treated similarly, except that a light petroleum extraction was not carried out. Stems were cut into approximately 15 cm lengths before extraction. Roots were washed quickly with cold water to remove stones and soil, and were shaken dry. An attempt to mince the roots was unsuccessful and they were extracted whole. Green fruit was minced, then extracted with approximately its own volume of aqueous ethanol (95% v/v).

Countercurrent distribution. The crude aqueous extracts, from which ethanol had been removed, were submitted to a five-transfer countercurrent distribution in six 5 l. separating funnels. Portions (about 2 l.) of each extract were placed in the first funnel, and equal volumes of water in the other five as the upper (stationary) phase. The extract was shaken with an equal volume of chloroform, the phases were allowed to separate, and the lower chloroform phase was transferred. The countercurrent distribution was continued by the Bush & Densen (1948) procedure in which successive extractions are made on each phase. Six chloroform phases were thus obtained ; these were combined and taken to small volume by rotary evaporation *in vacuo*. The chloroform solutions were examined for alkaloids by thin-layer chromatography in solvent system C but, except in the two root extracts which contained tomatidine, none was detected.

The aqueous phases, subsequently referred to as countercurrent Fractions 1, 2, 3, etc., were concentrated individually in a rotary evaporator at below 45° C. Aliquots of the fractions for each plant tissue were taken to dryness and the weight of dissolved solid obtained. These solutions were stored at 0° C, and chloroform was added to inhibit growth of moulds.

Identification of the active principle

The active principle was identified in extracts obtained from infected tomato leaves and these experiments are described in detail. Similar experiments were also done with extracts from other parts of infected plants and from healthy plants.

Fractionation on Sephadex. Sephadex G 25, medium grade (50 g), was screened by stirring with water (600 ml.), allowing the gel particles to settle for 10 min, and sucking off the supernatant suspension of fine particles. This was repeated until few particles remained in suspension. The column was filled by pouring the gel, as a slurry, into water. The dimensions of the column were 2.9 cm diameter by 33.5 cm long. It was regenerated between fractionations by washing with 0.2 N-sodium hydroxide solution, then with water.

The aqueous phase, designated countercurrent Fraction 1, from infected tomato leaves was fractionated on the column. A portion (10 ml., containing 129.6 mg/ml. of dissolved

solid) of this solution was eluted with water at 60 ml./hr. Fractions (approximately 9 ml.) were collected until all the pigment had been eluted ; this required 100 fractions. The contents of each tube were examined by chromatography, fractions pooled according to content, and each bulk solution was concentrated to 10 ml. by rotary evaporation *in vacuo*.

Preparation of an alkaloid-free extract. A portion (25 ml.) of crude leaf extract from infected tomato plants was acidified with acetic acid (2.5 ml.) heated at 70 to 75° C on a steam bath, and treated drop by drop with concentrated ammonia solution (specific gravity, 0.890) until alkaline to litmus. The somewhat gummy, brown precipitate, which was expected to contain any alkaloid present, was filtered off, and the filtrate submitted to a countercurrent distribution between water and chloroform.

Isolation of tomatine. A portion (50 ml.) of countercurrent Fraction 1 from infected leaf extract was heated at 75 to 80° C on a steam bath and brought to *p*H 9.3 by addition drop-by-drop of sodium carbonate solution (5 ml. 10%, w/v). The cold solution was filtered, and the yellow-brown precipitate was collected and dried. It was dissolved in hydrochloric acid (20 ml., approximately 1 N), reprecipitated with sodium carbonate solution, collected, and dried.

Crude alkaloid (253.4 mg) was dissolved in hot n-butanol saturated with water (15 ml.), the solution filtered, and the filtrate applied to a column (1.3 cm diameter by 8.4 cm long) of alumina (15 g). Alumina was deactivated by shaking with acetic acid (0.75 ml./400 g of alumina) ; it was then Brockmann grade 2 to 3. Elution was performed with butanol saturated with water, and fractions of about 4 ml. were collected. The contents of tubes 1 to 21 were combined, and partly concentrated to give tomatine as a flocculent solid (150 mg). After recrystallization from aqueous ethanol (75%, v/v), tomatine was obtained as a powder which gave a single spot on chromatography and did not separate on co-chromatography with authentic tomatine either on paper or on thin-layer plates. It had an infra-red spectrum identical with that of the pure alkaloid and had a melting point of 250 to 254° C (decomposition) on a Kofler hot-stage melting-point apparatus ; rate of heating, 4° C/min ; not preheated. Fontaine, Irving, Ma, Poole & Doolittle (1948) give a melting point of 263 to 267° C (decomposition) on a stage preheated at 250° C. (Found: N, 1.35% ; calculated for $C_{50}H_{83}NO_{21}$: N, 1.35%.) The dry alkaloid was hygroscopic and this caused difficulty with the analysis.

Quantitative estimation of tomatine

In preliminary experiments it was found that the method of Schulz & Sander (1957), in which tomatine is precipitated as a complex with cholesterol, gave somewhat erratic results with the comparatively crude plant extracts. Next, the photometric method for estimating

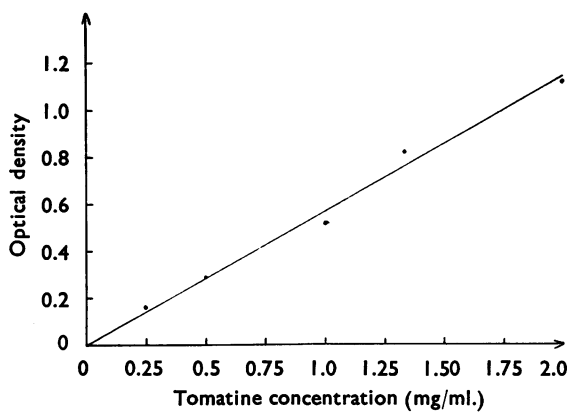

Fig. 2. Calibration curve for estimation of pure tomatine by the absorbence at 530 mμ of the colour developed with concentrated sulphuric acid and formaldehyde.

solanine (Rooke, Bushill, Lampitt & Jackson, 1943), a glycoside present in potato and with a structure similar to that of tomatine, was used. This method was also found to be unsatisfactory for tomatine since the colour yield was low and gave a nonlinear plot against concentration, even with the modified procedure of Baker, Lampitt & Meredith (1955). Reproducible results were, however, obtained by use of the following conditions.

A portion of plant extract, containing tomatine (about 10 to 20 mg), was heated at 70 to 80° C, and made alkaline to phenolphthalein by addition drop by drop of concentrated ammonia solution. The precipitate was collected, washed with dilute ammonia solution, dried, then dissolved in sulphuric acid (15 ml.; 1%, v/v) and diluted to 25 ml. with water. Concentrated sulphuric acid (5 ml.) was added slowly from a burette to an aliquot (2.5 ml.) of this solution with continuous shaking of the flask, followed by formaldehyde (2.5 ml.; 1%, v/v) added similarly. A purple-red colour developed. After 90 min the optical density was measured in a Unicam SP500 spectrophotometer at 530 mμ against a blank which contained water instead of a plant extract. The tomatine content of each solution was obtained by comparison with a calibration curve for pure tomatine (Fig. 2). Usually, a number of determinations at different concentrations were made for each extract.

RESULTS

Table 1 shows the difference in survival times of control guinea-pigs exposed to aerosols containing histamine acid phosphate in two concentrations, 0.6% as used previously (Broome *et al.*, 1962) and 1% used in this work.

TABLE 1

SURVIVAL TIMES OF CONTROL GUINEA-PIGS EXPOSED TO AEROSOLS CONTAINING HISTAMINE ACID PHOSPHATE

* Broome *et al.* (1962), 330 animals; † this paper, 418 animals

Survival time (min)	Animals (%) surviving aerosol with histamine concentration	
	0·6%*	1%†
<6	46	61
<8	72	81
>11	13	9
>20	3·6	3·1

Antihistamine activity was detected in the aqueous phase after countercurrent distribution of the crude plant extracts. No antihistamine activity was found in the chloroform layer. Most of the water-soluble material remained in the original solution (countercurrent Fraction 1) or passed into the first aqueous phase (countercurrent Fraction 2) on distribution. For instance, countercurrent Fraction 1 of the infected stem extract contained 53 g of dissolved solid, while countercurrent Fraction 2 contained 92.5 mg, and only 19 mg were present in the remaining aqueous phases. As shown in Table 2, there was considerable variation in the amount of water-soluble material, both between different parts of the plant and between healthy and infected tissues.

Identification of the active principle

The active principle was identified in the extract from crown-gall infected tomato leaves. A partial purification of the active compound was achieved by chroma-

TABLE 2

WATER-SOLUBLE MATERIAL OBTAINED FROM HEALTHY TOMATO PLANTS AND FROM THOSE INFECTED WITH CROWN-GALL

Tissues were extracted with aqueous ethanol (95%, v/v), the ethanol was evaporated, and the resulting solution was submitted to countercurrent distribution between water and chloroform. The percentage extracted was calculated from the total dissolved material in the aqueous phase

Plant tissue		Wet weight (kg)	Percentage extracted
Stems	Infected	18·10	0·29
	Healthy	1·10	4·57
Leaves	Infected	47·10	Not recorded
	Healthy	3·15	Not recorded
Roots	Infected	2·65	1·23
	Healthy	0·45	0·94
Green fruit	Infected	107·20	0·24
	Healthy	5·70	1·56

tography on Sephadex. This method had two advantages: first, the extract was recovered quantitatively; and second, since the only manipulation was elution of an aqueous extract with water, it was possible to test all the fractions obtained for antihistamine activity without chemical modification. The active constituent was located in a single peak.

The results of a typical fractionation are shown in Table 3. The contents of tubes 1 to 6 (forerun) from the columns containing Sephadex G25 were discarded. The other fractions varied progressively in colour from olive-yellow (tubes 7 to 12) to deep yellow (13 to 16), and then the colour decreased in intensity until Fraction 100 was colourless. After their contents had been chromatographed on paper, the fractions were pooled into four bulk-solutions, and concentrated. A sample (1 ml.) of each was taken to dryness. The distribution of dissolved solid is shown in Table 3. Fractions 7 to 12 contained compounds of high molecular weight (>3,500). These were probably pectic substances which do not enter the particles of the gel and are eluted first. Alkaloid was present in Fractions 13 to 19, and phenolic compounds (for example, flavonoid pigments) from Fraction 13 onwards with a peak at Fractions 21 to 31. Most of the solid was found in Fractions 13 to 21, which contained most of the low molecular weight constituents of the extract. The amino acids were slightly separated from the alkaloid on the column; they were also present in Fractions 22 to 39 which contained chlorogenic acid and

TABLE 3

FRACTIONATION ON SEPHADEX OF COUNTERCURRENT FRACTION 1 FROM EXTRACT OF LEAVES OF TOMATO PLANTS INFECTED WITH CROWN-GALL

Elution was carried out with water, fractions were pooled according to content and bulk solutions were concentrated to the original volume of the sample. The total solid recovered was 129·3 mg/ml., and the sample contained 129·6 mg/ml.

Tube number	Dissolved solid	
	(mg/ml.)	(% of total)
7–12	2·5	1·9
13–21	112·4	87·0
22–39	9·4	7·3
40–100	5·0	3·9

other, unidentified, phenolic compounds. Rutin was the main constituent of Fractions 40 to 100 and slowly crystallized out. Aromatic compounds in general tend to be retained on Sephadex (see Gelotte, 1960). This effect, particularly noticeable with the phenolic compounds in the tomato extracts, may be of general value in the study of plant extracts.

Fig. 3 (*a* to *d*) shows the results obtained with the four bulk-solutions, tested at a dose equivalent to 100 mg of dissolved solid in the original sample. Significant

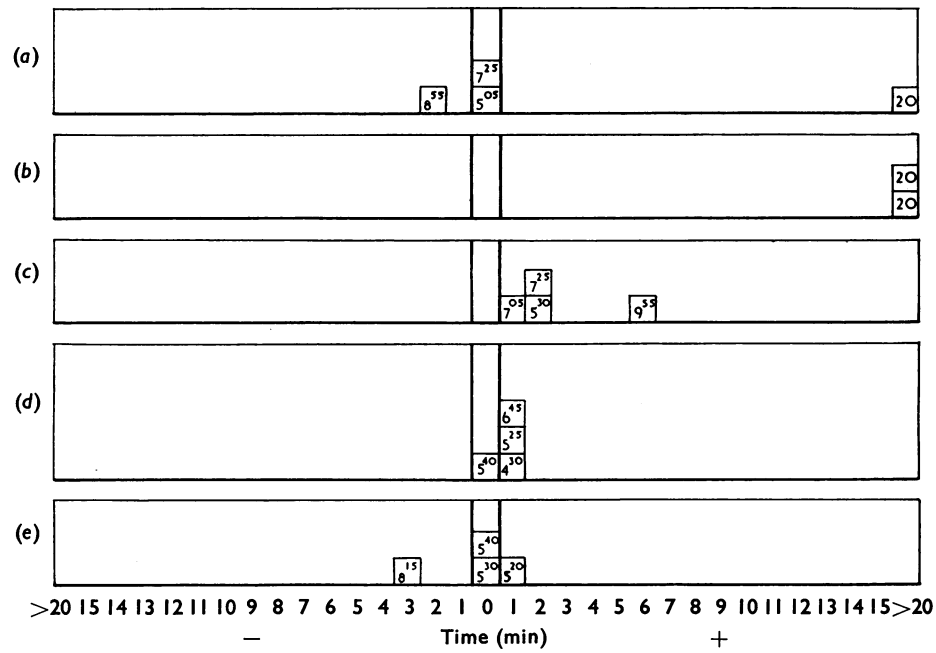

Fig. 3. Protection of guinea-pigs against a 1% aerosol of histamine acid phosphate by intraperitoneal injection of solutions from countercurrent Fraction 1 of infected tomato leaves. In each experiment the dose was equivalent to 100 mg of solid in the fraction. (*a*) to (*d*), after fractionation on Sephadex: (*a*), tubes 7 to 12; (*b*) tubes 13 to 21 (two animals died before the test); (*c*), tubes 22 to 39; (*d*), tubes 40 to 100; (*e*), countercurrent Fraction 1 after removal of alkaloid. The results in this and subsequent Figs. show the difference in survival time between the injected animal and its control. The difference is given as +, if the injected animal survived longer, as −, if the control did, and as 0, if the difference was 30 sec or less or if both animals survived 20 min. Each square represents one animal and the numbers in the square indicate the survival time (in minutes and seconds) of the longest surviving animal, except at zero time where mean values are given.

antihistamine activity was found to be associated with the alkaloid since activity was restricted to the solution from Fractions 13 to 21 (Fig. 3*b*), which was the only one which contained alkaloid. The inactivity of the solution from Fractions 40 to 100 (Fig. 3*d*) is of interest in view of a previous observation that rutin conferred slight protection on guinea-pigs against shock produced by injection of a lethal dose of histamine (Wilson, Booth & DeEds, 1951). As a check, rutin was tested

separately by the histamine aerosol technique at doses of 10 and 40 mg per animal. The results in Fig. 4 (*a* and *b*) show that no activity was obtained with either dose.

The association of antihistamine activity with the presence of alkaloid in the plant extract was confirmed by testing an alkaloid-free extract. Countercurrent distribution of infected leaf extract from which alkaloid had been precipitated gave a Fraction 1 which showed no alkaloid spot on the thin-layer chromatography in solvent C. Fig. 3*e* shows that guinea-pigs injected with this alkaloid-free solution (100 mg of dissolved solid) were not protected against the effects of the histamine aerosol.

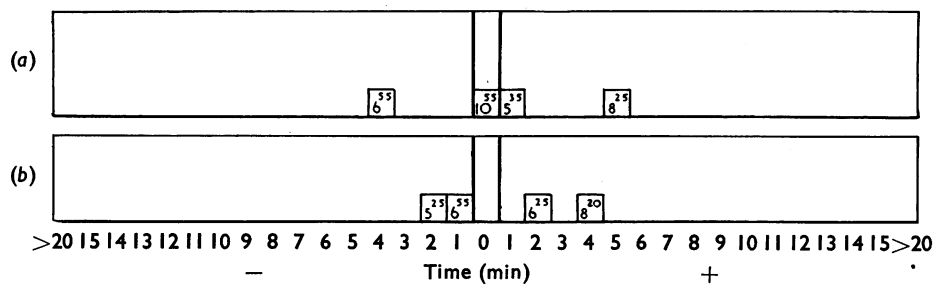

Fig. 4. Protection of guinea-pigs against a 1% aerosol of histamine acid phosphate by intraperitoneal injection of rutin: (*a*), 10 mg; (*b*), 40 mg. Details as in Fig. 3.

The alkaloid isolated from countercurrent Fraction 1 of infected leaf extract was identified as tomatine. Fig. 5*a* shows that protection for 20 min against the histamine aerosol was conferred on four guinea-pigs injected with 3 mg of the isolated tomatine.

Fig. 5 (*b* to *d*) shows the results obtained with guinea-pigs injected with authentic tomatine (3, 2.5 and 2 mg, respectively). Strong protection was conferred by 3 mg but slight protection was also observed by 2 mg.

Tomatine was the only alkaloid detected by thin-layer chromatography in every extract which showed antihistamine activity. Active extracts were obtained from all parts of both infected and healthy tomato plants. Table 4 gives, for countercurrent Fractions 1 and 2 of the extracts from infected and healthy leaves, the

TABLE 4

PROTECTIVE DOSE AND TOMATINE CONTENT OF COUNTERCURRENT FRACTIONS FROM TOMATO LEAVES

The minimum protective dose is the amount required to protect guinea-pigs against the lethal effects of a 1% aerosol of histamine acid phosphate

Extract	Tomatine content (%)	Minimum protective dose of: Total solid (mg)	Minimum protective dose of: Tomatine (mg)
Infected			
Countercurrent fraction 1	4·65	100	4·65
Countercurrent fraction 2	11·15	31·4	3·5
Healthy			
Countercurrent fraction 1	3·7	100	3·7
Countercurrent fraction 2	12·0	31·6	3·8

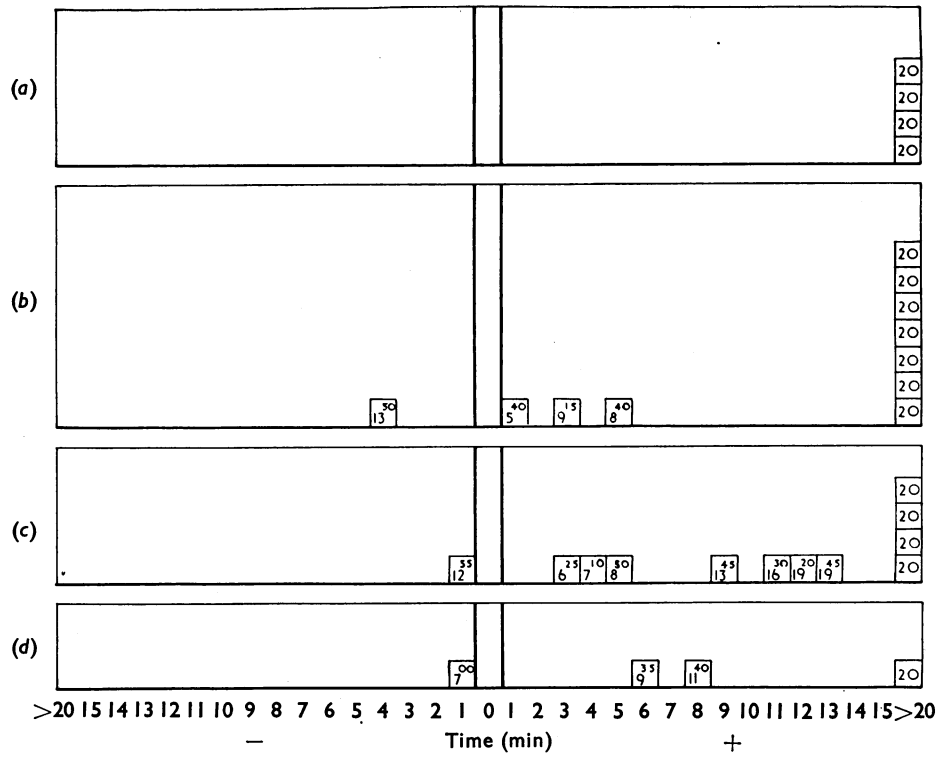

Fig. 5. Protection of guinea-pigs against a 1% aerosol of histamine acid phosphate by intraperitoneal injection of tomatine. (*a*), isolated from countercurrent Fraction 1 of infected tomato leaves, 3 mg; (*b*), pure tomatine, 3 mg; (*c*), pure tomatine, 2.5 mg; and (*d*), pure tomatine, 2 mg. Details as in Fig. 3.

amounts of solid that gave protection, the percentages of tomatine, and, calculated from this, the weights of tomatine injected. These weights are sufficient in each instance to account for the activity observed. The values for all four extracts are equal within narrow limits and afford further presumptive evidence that tomatine is the only active material present, and that no difference exists between infected and healthy plants.

Properties of extracted tomatine

The tomatine content of the extracts appeared to decrease on storage. This decrease would account for the loss in antihistamine activity observed in stored extracts; this loss has been observed previously (Broome *et al.*, 1962). Tomatine may be unstable in the plant extracts, or it may be destroyed by an enzyme (or enzymes), extracted from the tomato, with weak activity at low temperature as has been observed in stored vegetable tissue (for example, Joslyn, 1949). The extracts were susceptible to microbial contamination and subsequent decomposition, and despite suitable precautions this occurred in a few instances. These decomposed

extracts also showed a loss of activity. The mode of decomposition of tomatine was not studied, but a stock solution in sulphuric acid (1%, v/v) slowly decomposed.

Toxicity of extracts

Guinea-pigs which had been injected with extracts containing sufficient tomatine to confer subsequent protection against the histamine aerosol usually showed side-effects which included diarrhoea, loss of righting reflexes, decrease in body temperature, and ruffling of coat. Some animals also seemed comatose and appeared partly anaesthetized. These effects varied in severity from animal to animal. Guinea-pigs injected with large doses of plant extracts (containing about 5 mg of tomatine or more) died before test in a number of experiments.

Tomatine may also exert a delayed toxic effect in the guinea-pig. About one-quarter of the injected animals died during the 3 days after exposure to the histamine aerosol. None of the controls which survived the first test with the aerosol died during this period.

Long-term protection against histamine aerosol

Guinea-pigs which survived exposure to the histamine aerosol for 20 min were re-exposed 3 or 4 days later. Fig. 6*a* shows the results of retest of all control animals, and Fig. 6*b* the results for animals which had been injected with plant extracts or with pure tomatine. There may be a slight protection in the injected animals: fifty-three gave positive results and twenty-one negative, as opposed to

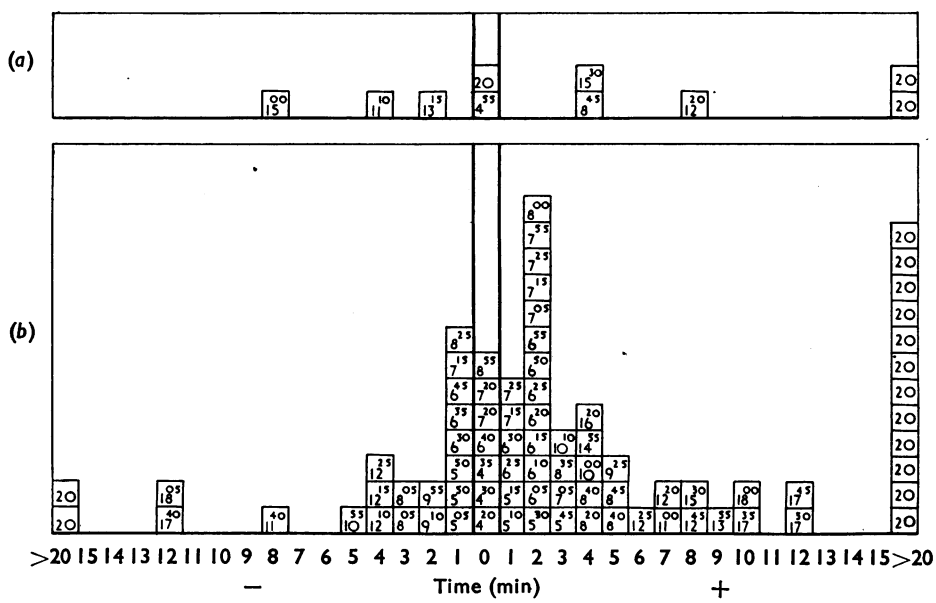

Fig. 6. Protection of guinea-pigs against a 1% aerosol of histamine acid phosphate on retest. All animals had survived one exposure to the aerosol for 20 min. (*a*), control animals; (*b*), animals injected intraperitoneally with either tomatine or a plant extract containing tomatine. Details as in Fig. 3.

five positive and three negative for the controls. No firm conclusion can be deduced from these figures because of the small number of surviving controls. Nine of the twelve injected animals which survived a second exposure to the aerosol also survived a third ; the corresponding result for the controls was one of three. This surviving control died on its fourth test, while the single injected guinea-pig which survived a fourth and fifth test died on the sixth test, 18 days after injection.

Antihistamine activity of pure steroid alkaloids

Tomatidine. This is the aglycone of tomatine and was present in the chloroform solution after countercurrent distribution of the root extracts from both healthy and infected tomatoes. Fig. 7*a* shows that no protection was conferred on guinea-pigs

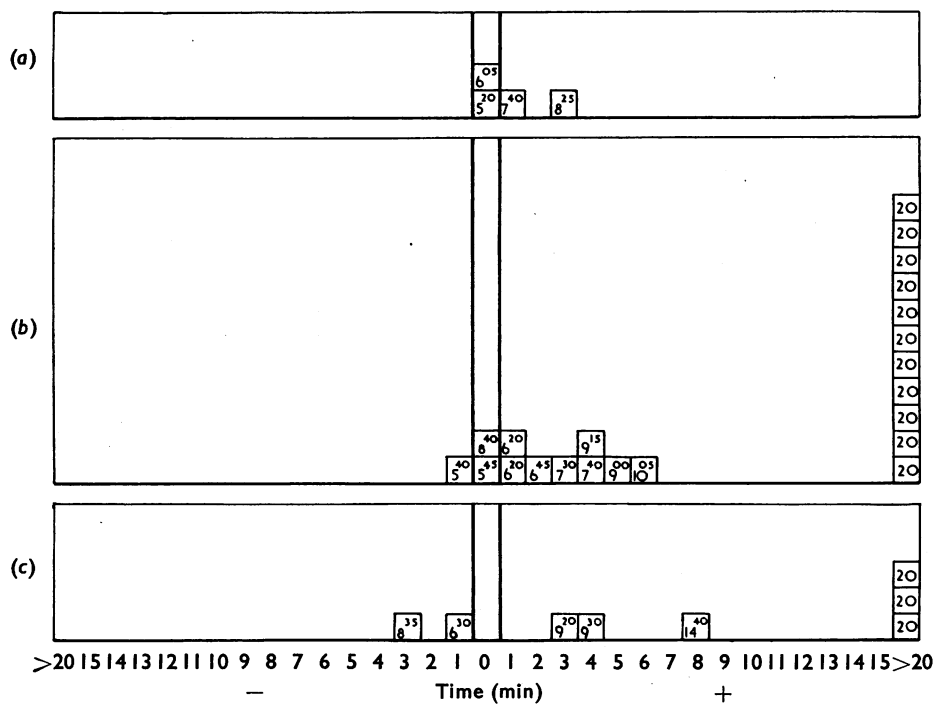

Fig. 7. Protection of guinea-pigs against 1% aerosol of histamine acid phosphate produced by intraperitoneal injection of steroid alkaloids. (*a*), Tomatidine, 2 mg; (*b*), solanine, 4 mg; and (*c*), solasonine, 6 mg. Details as in Fig. 3.

injected with tomatidine (2 mg, equivalent to 4.97 mg of tomatine). Since this amount of tomatidine was ineffective, it is probable that the presence of sugar residues in the molecule is necessary for antihistamine activity.

Solanine. This is related to tomatine but is not present in the tomato. It consists of a branched trisaccharide chain, containing one molecule each of rhamnose, galactose and glucose, attached to a steroid nucleus. The steroid portion of the molecule has rings E and F fused with a bridge-head nitrogen atom. Fig. 7*b*

shows that 4 mg of solanine had antihistamine activity similar to that of 3 mg of tomatine.

Solasonine. This contains the trisaccharide unit present in solanine, attached to a steroid nucleus resembling tomatidine but with a 5,6 double bond and the inverse configurations at C-22 and C-25. Fig. 7*c* shows that some protection against the histamine aerosol was conferred by injection of solasonine (6 mg). The effect was not as pronounced as with solanine (4 mg) or tomatine (3 mg).

DISCUSSION

The pharmacological properties of tomatine have been studied in several species of animals (Wilson, Poley & DeEds, 1961) but not previously in guinea-pigs, nor was the alkaloid administered by intraperitoneal injection as in our experiments. The drop in blood pressure associated with intravenous injection of tomatine is reminiscent of the action of the closely-related veratrum group of steroid alkaloids. The side-effects observed in guinea-pigs treated with tomatine are similar to the central effects described previously for tomato extracts (Broome *et al.*, 1962) and resemble the symptoms of solanine poisoning in animals which have eaten green potatoes (for example, Forsyth, 1954). No cases of tomatine poisoning appear to have been described. The difference in intensity of the central effects, observed by Broome *et al.* (1962) and caused by saline or myristate solutions of material from tomato plants infected with crown-gall, can probably be attributed to the difference in solubility of tomatine under these conditions.

The identification of tomatine as the compound responsible for the antihistamine activity of extracts from tomato plants has revealed a new type of biological action of the alkaloid. The steroid alkaloids have a variety of pharmacological actions (see review by Alauddin & Martin-Smith, 1962) and the present results seem to indicate that protection of guinea-pigs against the effects of a histamine aerosol is a general property of glycosides based on the solanidine and spirosolane skeletons. Our results suggest that this protection is short-lasting and of theoretical rather than practical interest. It seems likely that the steroid alkaloids studied have to be added to a rather heterogeneous collection of alkaloids found by Herxheimer (1956) to exert an antagonistic effect on the bronchoconstrictor action of a histamine aerosol in guinea-pigs.

Extracts of tomatoes infected with crown-gall exerted no antihistamine activity when added to an organ-bath containing a guinea-pig isolated ileum preparation (Broome *et al.*, 1962). Pure tomatine (50 μg) was also found to be without activity in this respect, in contrast to mepyramine (0.4 μg) which causes total inhibition, followed by subsequent recovery (N. G. Waton, personal communication). This result suggests that protection observed in the intact animal is not a direct effect of the tomatine but may be caused either by a metabolite or, indirectly, as a result of a nonspecific (irritant) effect of the alkaloid.

The metabolism of cardiac glycosides such as digitoxin appears to be a combination of hydroxylation, hydrolysis of the glycoside linkage, and conjugation followed by excretion in the urine (Martin & Wright, 1960), and may not differ between species (Ashley, Brown, Okita & Wright, 1958). The metabolism of tomatine has not

been studied, but it might well proceed along a similar pathway. Since the aglycone tomatidine is inactive, a metabolite of this type produced by hydrolysis of the glycoside is probably not involved in the antihistamine activity.

We are indebted to our colleague Professor W. Feldberg for valuable discussion of this work while it was in progress and when this paper was being prepared for publication. We should like to thank the Glasshouse Crops Research Institute for growing the tomatoes and Mr J. P. Stean for assistance with the animal testing.

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