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HISTAMINE PROTECTION PRODUCED BY OAK GALL EXTRACTS

BY

D. H. CALAM

From the National Institute for Medical Research, Mill Hill, London N.W.7

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The antihistamine activity of extracts from oak galls was first noted in Hungary (Kovacs & Szabadi, 1950; Kovacs, Kovacs, Szabadi & Varsányi, 1952) following the observation of similar activity in tannic acid prepared from such galls (Gyüre & Kovacs, 1949). These findings were later confirmed (Feldberg & Kovacs, 1960) and stable extracts obtained (Broome, Callow, Feldberg & Kovacs, 1962). Alcoholic extracts of oak galls also conferred protection on guinea-pigs against an aerosol of 5-hydroxytryptamine (Berry, Holgate & Lockhart, 1962).

The original intention behind the experiments described here was to isolate the active principle from a fresh supply of galls. The results, however, indicate that although there appears to be significant antihistamine activity in the oak gall extracts, these extracts are also toxic, the activity is only of short duration and probably a non-specific rather than specific effect. Further attempts to isolate the active principle have been abandoned.

METHODS

The method used was that of Calam & Callow (1964) in which a treated animal and its control are simultaneously exposed, 4 hr after injection, to an aerosol of 1% histamine acid phosphate until respiration ceases or for a maximum of 20 min. Material from chloroform-methanol extracts was dissolved in isopropyl myristate for injection. Material from aqueous and ethanolic extracts was dissolved in saline, 0.9% (w/v). Control animals were injected with equal volumes of the corresponding solvent. Groups of four animals were tested with each extract except in preliminary tests when only two animals were tested at each dilution.

Preparation of extracts

Oak galls. Some confusion has unfortunately arisen in the literature about the species of galls used. Kovacs & Szabadi (1950) did not name their galls, while Kovacs *et al.* (1952) worked with "the two commonest galls: leaf and acorn galls of *Cynips quercus calicis.*" The galls used at Mill Hill were described as those produced by *Cynips quercus calicis* by Feldberg & Kovacs (1960) and those produced by *Andricus quercus-tozae* Bosc. by Broome *et al.* (1962). Berry *et al.* (1962) adopted this identification and also gave *Andricus quercus-tozae* Bosc. as the vector producing their galls.

The situation has been clarified with the aid of staff of the Hungarian Board of Woods and Forests, and Dr L. Moczar of the Hungarian National Museum, Budapest. The galls used in previous work at Mill Hill were immature and mature specimens produced by *Cynips hungarica* Htg. known as Magyar galls (Magyar gubacs). Examination of samples (kindly supplied by Dr. I. M. Lockhart) suggests that the galls used by Berry *et al.* were also mostly produced by *Cynips hungarica.*

These occur on oak leaves and are the most frequent in occurrence of all Hungarian oak galls. The galls of *Andricus quercus-tozae* Bosc. are similar in size and hardness but are a deeper red-brown colour, smoother with a characteristic ridged "collar" and are of comparatively rare occurrence. The galls produced by *Cynips quercus calicis* Burds. on acorn cups (one or two galls growing between the acorn itself and the cup) are also of frequent occurrence. These galls are smaller and somewhat softer than the Magyar galls and have a wrinkled, waxy surface, hence the common name fatty galls (Zsiros gubacs).

The galls used in the present investigation were collected in Southern Hungary in the autumn of 1964 and early in 1965 and were of the two kinds described: Magyar galls and fatty galls. The galls were selected and those that were mouldy or blown (through escape of the insect) were discarded.

Chloroform-methanol extracts. These were prepared as described previously (Broome *et al.*, 1962) and taken to dryness in a rotary evaporator *in vacuo* below 40°. The yield of dark green waxy solid varied between 0.26 and 0.70% from Magyar galls and between 3.75 and 7.28% from fatty galls. The weight of extract from Magyar galls injected intraperitoneally into guinea-pigs was 26 to 280 mg in 2 to 4 ml. isopropyl myristate and from fatty galls was 111 to 728 mg in 2 or 3 ml.

Aqueous extracts. Ground galls were stirred with water (10 to 20 ml./g gall) at room temperature for 2 hr under argon. The extracts were filtered and the brown filtrates concentrated *in vacuo* below 40°. Aliquots were taken to dryness and showed a yield of 8% from Magyar galls and 15% from fatty galls. These extracts were injected as saline solutions (1 to 4 ml.) containing 4 to 64 mg solid from Magyar galls and 30 to 60 mg solid from fatty galls.

Ethanol extracts. Extractions by the method of Feldberg & Kovacs (1960) were performed under argon and extracts taken to dryness *in vacuo* below 30°. The yields of dark brown solid were between 8 and 22% from Magyar galls, 24.65% from fatty galls. Refluxing the gall powder for 5 min with ethanol (10 mg/g) under argon gave yields of 12.6 and 14.8% from Magyar galls, 24.6 to 29.6% from fatty galls. Cold extraction, by the method described for aqueous extracts, yielded 6.4% from Magyar galls, 25.6 from fatty galls.

Large scale extractions with several kg of galls were prepared by Soxhlet extraction with 95% ethanol for 3 hr. The extracts were taken to dryness and the residues (about 350 g each) re-extracted 4 times with portions of chloroform (500 ml.) for 2 hr under reflux. The yield of chloroform-insoluble solid was 9.3% from Magyar galls, 13.1% from fatty galls. A small scale experiment with fatty galls gave 23.6% solid.

Guinea-pigs were injected intraperitoneally with 12.5 to 100 mg (mostly 25 to 50 mg) solid from Magyar gall extracts dissolved in 1 or 2 ml. saline, with 25 to 50 mg (mostly 35 mg) in 2 ml. from small scale fatty gall extracts and with 37.5 to 100 mg solid from large scale fatty gall extracts in 1 to 4 ml. saline.

Partial purification of ethanol extract. A tannin-enriched fraction was prepared from an ethanol extract of fatty galls. The extracted solid was refluxed for 1 hr with ether. Insoluble material (4.56 g) was dissolved in 0.1 M sodium dihydrogen phosphate (100 ml.) and the solution extracted with portions of ethyl acetate (100 ml.) until the extract no longer gave a colour with ferric chloride. The ethyl acetate extracts were combined and evaporated to dryness *in vacuo* below 30°. The residue was dissolved in water and the extraction repeated. The resulting solid was dissolved in water (10 ml.) and freeze-dried to give a tannin-enriched fraction (0.37 g from 20 g galls) as a cream-coloured powder. Guinea-pigs were injected with 39 mg in 2 ml. saline.

A sample of commercial tannic acid (Hopkin and Williams Ltd.) was similarly purified and guinea-pigs injected with 12 to 40 mg in 2 ml. saline.

RESULTS

The response of control guinea-pigs to a 1% histamine aerosol has been reported previously (Calam & Callow, 1964). There appeared to be a seasonal variation in sensitivity, reflected in the number of controls surviving 20 min exposure. Although

comparable numbers were tested, more survived during the early part of the year than in the summer and the number was twice as high in January as in any other month. The cause of this variation was not traced. Controls were generally affected by the aerosol faster than were the test animals.

Chloroform-methanol extracts. The only side-effect noted after injection of Magyar gall extracts was slight abdominal distension. Higher doses of fatty gall extracts produced this effect together with ruffling of the coat, while animals injected with the highest dose (728 mg) were initially comatose but soon recovered. No difference in response to the aerosol was noted with either type of extract over a wide range of concentrations. The results for all the tests with each gall extract are given in Fig. 1. Six animals tested with each extract and five of their controls survived the first exposure to the aerosol. The

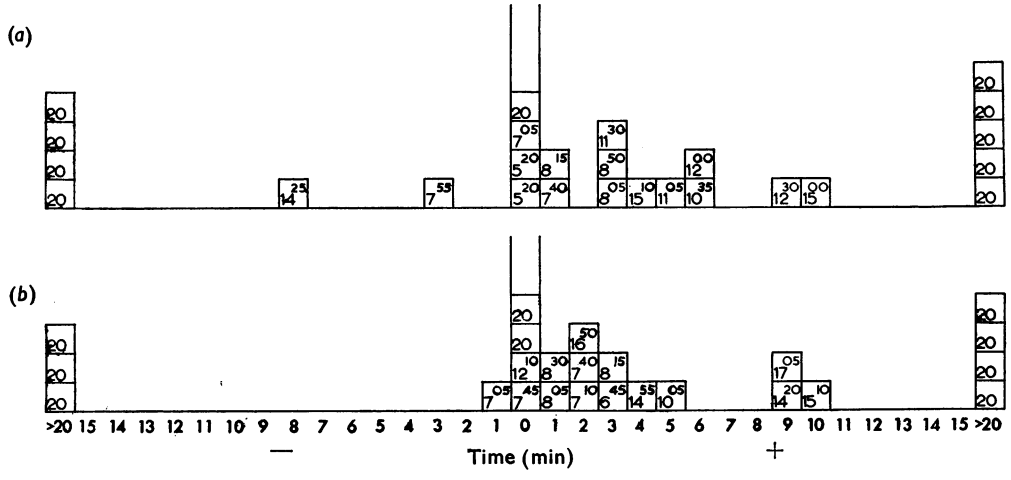

Fig. 1. Protection of guinea-pigs against a 1% aerosol of histamine acid phosphate by intraperitoneal injection of chloroform-methanol extracts of (*a*) Magyar oak galls; (*b*) fatty oak galls. This and the subsequent figures show the results of exposure to the aerosol as a difference in survival time between the treated animal and its control. This difference (in min) is given on the horizontal scale 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 exposure. Each square represents one test and the numbers in the square indicate the survival time (min and sec) of the longest surviving animal, except at zero time, where mean values are given.

test animals were injected with 40, 50 (2), 256 (2) and 280 mg Magyar gall extract and with 111, 175.5 (2), 218 and 728 (2) mg fatty gall extract.

Aqueous extracts. Extracts prepared from both Magyar and fatty galls produced the same effects on injection as the chloroform-methanol extracts. One guinea-pig injected with 60 mg fatty gall extract died before test. There was no apparent difference in the degree of protection conferred by different amounts of the extracts. The results of all tests are given in Fig. 2. No controls survived the full 20 min in this group, the surviving test animals had been injected with 4, 16 and 64 mg Magyar gall extract and 60 mg fatty gall extract.

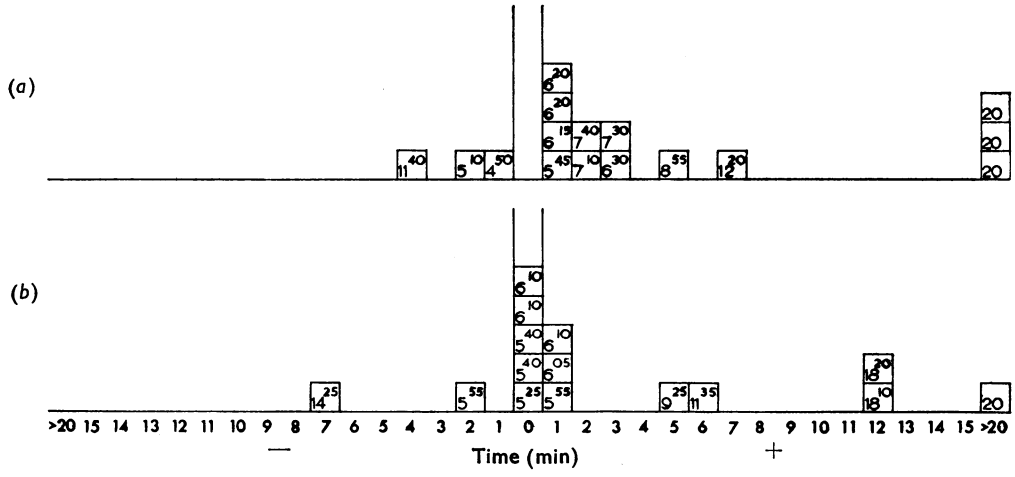

Fig. 2. Protection of guinea-pigs against a 1% aerosol of histamine acid phosphate by intraperitoneal injection of aqueous extracts of (*a*) Magyar oak galls; (*b*) fatty oak galls. Details as in Fig. 1.

Ethanol extracts. These produced the same side-effects on injection as the other extracts, together with some loss of righting reflex. Some guinea-pigs died before test when injected with 44 mg or more Magyar gall extract: half died with 70 mg and all died with 100 mg. About 8% of guinea-pigs injected with fatty gall extracts died before test, but this effect was not apparently related to the weight of solid injected. Both Magyar and fatty gall extracts exerted some degree of protection against the histamine aerosol, but this did not increase significantly with increased doses. Figs. 3,*a*, *b* and *c* show the results obtained with 25, 40 and 50 mg Magyar gall extract. The positive results at 25 and 50 mg are similar, while the effect at 40 mg is much less pronounced. Fig. 3,*d* shows the results with 35 mg fatty gall extract: the protection is comparable with that obtained with Magyar extracts.

No significant activity was detected in large scale extracts below doses which were lethal for some guinea-pigs.

Partially purified ethanol extract. Fig. 4 shows that no activity was observed after injection of the tannin-enriched fraction (39 mg) and, at most, slight protection by 40 mg tannic acid.

Prolonged protection. The percentages of all injected animals surviving a first exposure to the aerosol were 5% for controls, 17% for Magyar gall extracts and 15% for fatty gall extracts. The oak gall extracts, however, exert a delayed toxicity; over 70% of animals injected with Magyar gall extracts which survived the first exposure, and over 80% of those injected with fatty gall extracts, died before a second exposure three or four days later. Few deaths occurred on the day of the first test. In contrast, the corresponding controls which died before a second test died soon after the first test from the effects of the histamine aerosol. The delayed toxicity was most marked with ethanol extracts, less with chloroform-methanol and aqueous extracts. Fig. 5 shows results obtained for all guinea-pigs exposed to the aerosol a second time. Four animals survived a second exposure, the two injected with fatty gall extracts died before a third

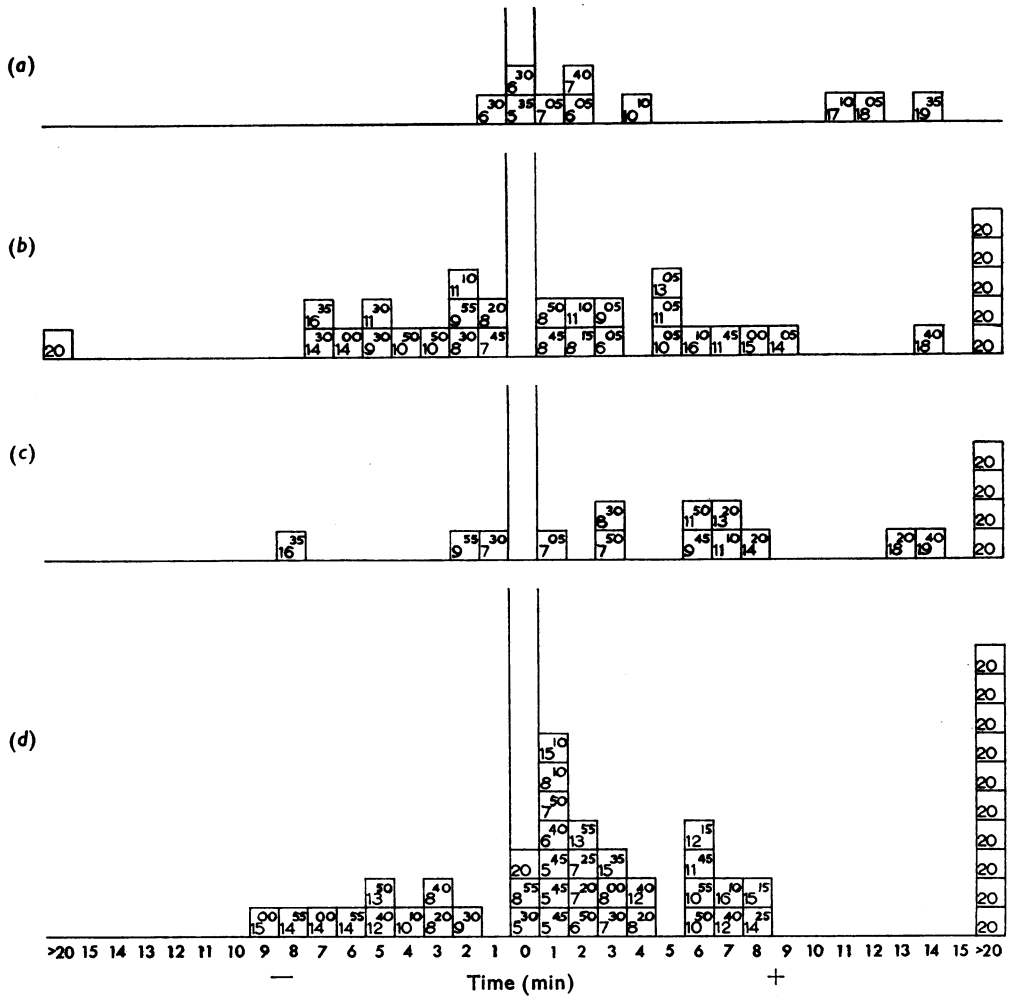

Fig. 3. Protection of guinea-pigs against a 1% aerosol of histamine acid phosphate by intraperitoneal injection of ethanol extracts of Magyar oak galls: (*a*) 25 mg; (*b*) 40 mg; (*c*) 50 mg; and of fatty oak galls: (*d*) 35 mg. Details as in Fig. 1.

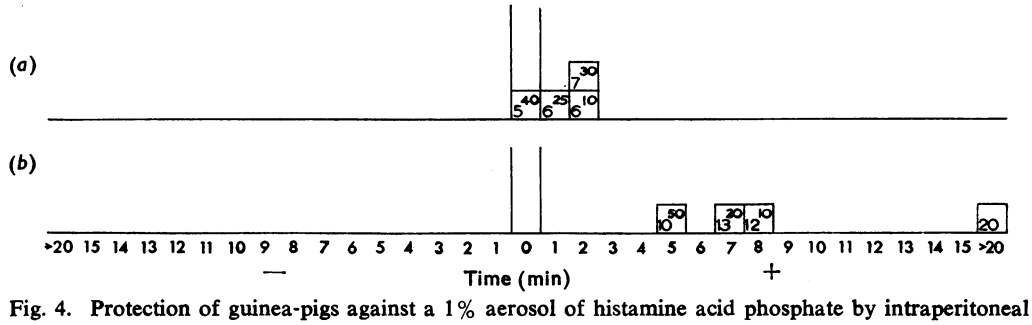

Fig. 4. Protection of guinea-pigs against a 1% aerosol of histamine acid phosphate by intraperitoneal injection of (*a*) tannin-enriched fraction from fatty oak galls, 39 mg; (*b*) tannic acid, 40 mg. Details as in Fig. 1.

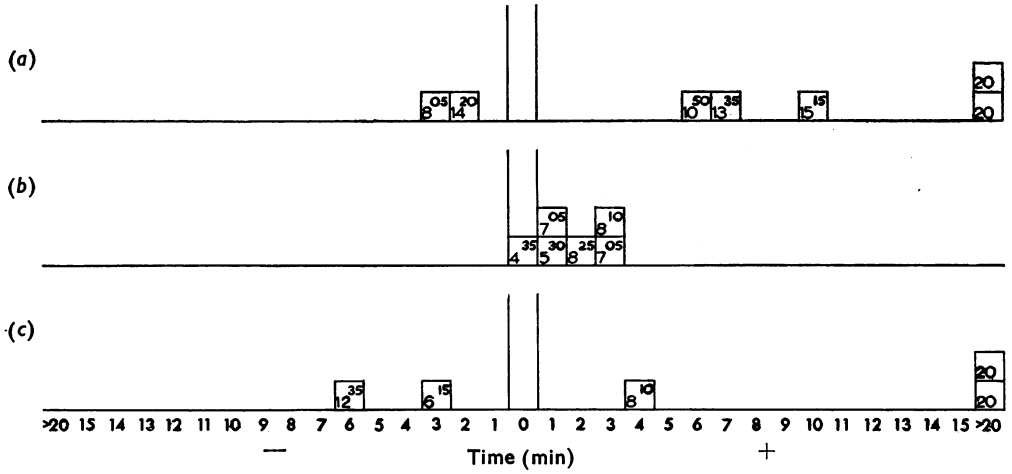

Fig. 5. Protection of guinea-pigs against a 1% aerosol of histamine acid phosphate on retest. All animals had survived one 20 min exposure to the aerosol. (*a*) Control animals; animals injected intraperitoneally with (*b*) Magyar oak gall extracts; (*c*) fatty oak gall extracts. Details as in Fig. 1.

exposure, one control died on its third test and the other on its fifth. No significant prolonged protection is therefore observed.

DISCUSSION

The results presented here confirm earlier observations that extracts of oak galls confer some protection on guinea-pigs against the effects of a histamine aerosol. The immediate protection is significant but slight in extent, and seems to bear no definite relation to the type of oak gall, the type of extract or the amount of extract injected. In view of the observation of Berry *et al.* (1962) that protection is also conferred against a 5-hydroxytryptamine aerosol, it seems likely that the antihistamine effect is non-specific in origin, being a secondary effect related only to the reaction of the guinea-pig to the toxicity of the injections, and not to a specific antihistamine.

Under the conditions employed, tannic acid was found to possess only slight antihistamine activity and tannin-enriched material from the galls was inactive. No further investigation of tannins was therefore made. This may be contrasted with the results of Gyüre & Kovacs (1949), who observed protection against an 0.4% histamine aerosol in guinea-pigs injected with 20–30 mg/kg tannic acid.

The protection is not prolonged. The results on second exposure to the aerosol are essentially the same for both control and test animals. This again suggests a non-specific effect.

The oak gall extracts are toxic, both immediately after injection, when minor side-effects are noted and higher doses are lethal, and over a period of three to four days, when a high proportion of test animals die having survived one test. The toxicity of tannic acid is well documented, and the presence of tannins and other polyphenolic compounds may explain the greater toxicity of ethanolic oak gall extracts.

In view of the results obtained, no further attempt has been made to isolate an antihistamine principle from oak galls.

SUMMARY

1. Several types of extract have been prepared from two species of oak gall. These extracts, injected intraperitoneally into guinea-pigs, conferred significant but transient protection on the animals against a 1% histamine aerosol. The extracts also displayed variable toxicity.

2. The antihistamine effects observed are of little value and no active principle has been isolated.

I am indebted to Dr R. K. Callow for valuable advice and discussion and to Mr J. Sitkey of the Hungarian Board of Woods and Forests for help in arranging the supply of oak galls. I should also like to thank Mr J. P. Stean for carrying out the animal tests.

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