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# HISTAMINE PROTECTION IN GUINEA-PIGS PRODUCED BY PLANT TUMOUR EXTRACTS

BY

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Stable extracts were obtained from plant tumours, such as Hungarian oak galls and crown galls of infected tomato plants, which, injected intraperitoneally into guinea-pigs, protected the animals against a subsequent histamine aerosol. The protection produced by the oak gall extracts lasted for a few days and that produced by the crown gall extracts, if injected in sufficient amounts, sometimes for a few weeks.

Extracts of plant tumours, such as Hungarian oak galls and crown galls of infected tomato plants, when injected intraperitoneally into guinea-pigs protect the animals against a subsequent histamine aerosol. This was first shown by Kovacs & Szabadi (1950) and by Kovacs, Kovacs, Szabadi & Varsanyi (1952). The experiments with oak galls were recently confirmed by Feldberg & Kovacs (1960), but no stable extracts could be obtained. In the present paper a method is described by which this became possible, and the method has been applied successfully also to the preparation of extracts from crown gall tumours. Injected into guinea-pigs, these extracts produced not only protection against a histamine aerosol but also strong effects on the central nervous system and on the digestive tract.

## METHODS

### *Exposure of guinea-pigs to histamine aerosol*

Guinea-pigs of both sexes weighing between 160 and 390 g were exposed in a chamber to a 0.6% histamine diphosphate aerosol produced by compressed air at a pressure of 4 atmospheres as described elsewhere (Feldberg & Kovacs, 1960). The maximal time of exposure was 20 min. If respiration ceased earlier the time of the last respiratory movement was recorded. Cessation of respiration usually followed a pattern in which the terminal respiratory movements consisted of small increasingly frequent movements of the auxiliary muscles of the nose. The animals were left in the chamber for at least 15 sec after the last of these movements to be certain that respiration had ceased. Without artificial ventilation the animals did not recover. When there were no terminal nose movements the animals were left in the chamber for at least 20 sec after the last respiratory movement. They also did not recover without artificial ventilation. With artificial ventilation some of the animals of both groups could be revived and subsequently re-exposed to a histamine aerosol. Artificial ventilation was given by blowing air into the lungs through a glass tube that had a curve at one end to facilitate entry into the animal's throat. The air was blown into the lungs rhythmically, about once every sec, 10 to 15 times, whilst the upper part of the stomach was compressed

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by hand so as to exclude accumulation of air in the digestive system. Such short periods of artificial ventilation, repeated a few times, often succeeded in breaking through the bronchospasm and re-establishing the sensitivity of the respiratory centre.

Since the sensitivity of guinea-pigs to the histamine aerosol varied greatly, the following method was used to determine the efficacy of extracts in producing protection. An injected animal and a control animal were together exposed to the histamine aerosol in the same chamber. The time of cessation of respiration for both animals was recorded, and the difference between their survival times determined. In Figs. 5, 6 and 7 this difference was plotted for each test as a square, on the left of a zero point if the control, and on the right if the injected animal survived longer. The squares above the number 1 indicate a difference in survival time of between 30 and 90 sec; the squares above the number 2, between 90 and 150 sec; and so on. If either the control or the injected animal survived the 20 min period of the aerosol, the square is given above the >20. The numbers inside the squares give the actual survival times either for the control, on the left of zero, or for the injected animal, on the right.. The squares above zero indicate either that both animals survived the 20 min exposure or that the difference in survival time was less than 30 sec, in which case the numbers inside the squares are the mean of the two survival times.

The same method of plotting was applied to the experiments of Fig. 4, in which control guinea-pigs were re-exposed to the histamine aerosol. Here the difference in survival time between the first and second exposures was plotted as a square on the left of the zero point if the survival time was longer during the first, and on the right if it was longer during the second exposure.

*Preparations of extracts from plant tumours*

Hungarian and English oak galls were used. The Hungarian oak galls were identified, by Mr. T. Quinlan, of the British Museum (Natural History), as those of *Andricus quercus-tozae*, Bose. The English oak galls, gathered locally, were the marble gall, of *Cynips kollari*, Hartig. The difference between the Hungarian and English oak galls is illustrated in Fig. 1. The galls were selected. Those which were blown due to the escape of the insect and those which were mouldy were discarded. The galls were ground in a beater cross grinding mill and extracted with 10 ml. chloroform-methanol (2 to 1) per gram powder. The mixture was saturated with argon, kept in stoppered flasks at room temperature under argon for 1.5 hr, and then filtered. The filtrate, a green solution which contained the active principle, was evaporated to dryness on a water bath (between 40 and 50° C) *in vacuo* under an argon

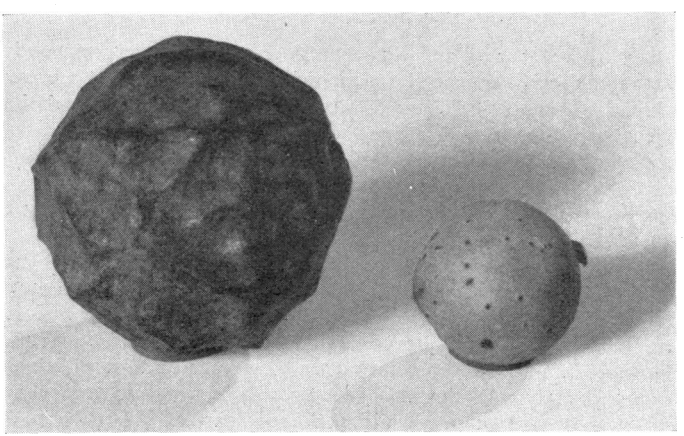

Fig. 1. Photograph of Hungarian oak gall (diameter 3.5 cm) on the left, and of English oak gall (diameter 1.9 cm) on the right.

stream. The dry residue was treated with 2 to 3 ml. chloroform (Reagent grade) per gram gall powder and filtered. The filtrate, which contained the active principle, was evaporated to dryness, again under reduced pressure on a water bath (between 40 and 50° C) and under an argon stream. The greenish residue no longer contained detectable amounts of tannin, but contained chlorophyll. It was stored at −5° C under argon in flasks closed with well-greased stoppers. The amount used for each intraperitoneal injection was dissolved in 2 to 3 ml. isopropyl myristate.

*Crown galls.* Crown gall tumours were produced by artificial infection with *Agrobacterium tumefaciens* (Strain East of Scotland College of Agriculture, culture no. 25, inoculated into tomato variety " Best Of All ") of tomato plants grown in the greenhouse at the Agricultural Research Council Plant Growth Substance and Systemic Fungicide Unit, Wye College. The freshly harvested infected tomato plant stems were put in dry ice until required for extraction. Fig. 2, which was kindly given to us by Professor Wain, shows two heavily infected plants

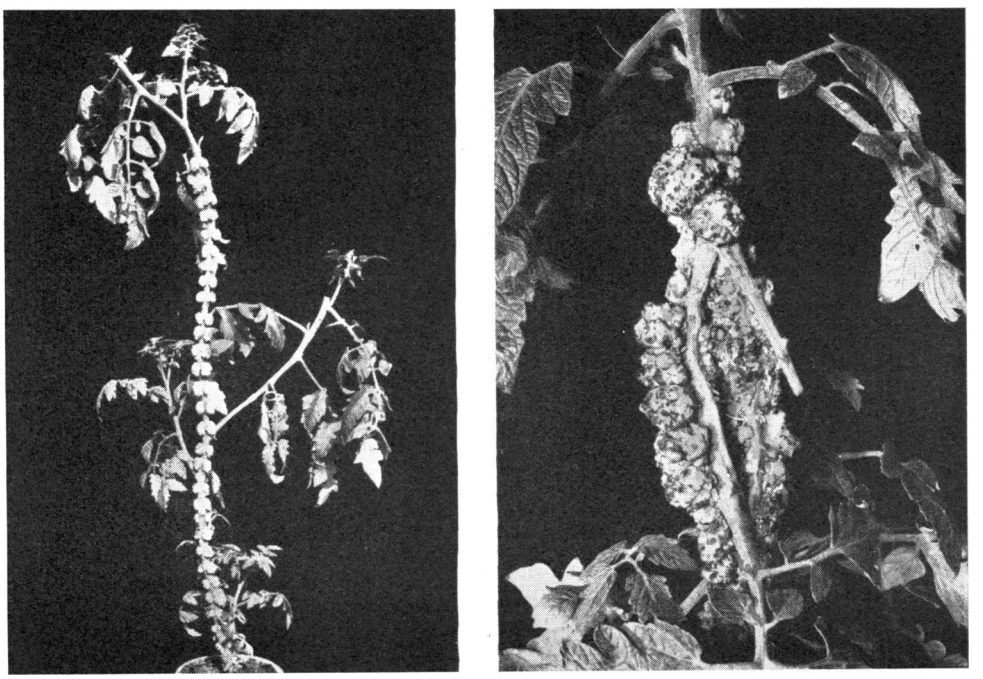

Fig. 2. Photograph of crown gall tumours on two tomato plants infected with *Agrobacterium tumefaciens*.

similar to those from which the frozen stems were taken. Usually whole infected stems together with the tumours were used. The stems were thawed, cut into small pieces with a razor and finely ground in a mortar with some acid-washed quartz sand and 2 ml. of hot ethanol (65° C) per gram tissue. The pulp obtained was heated in a beaker to 65° C for 5 min, filtered on a Büchner filter, and the mince was re-extracted with 1 ml. hot ethanol per gram tissue. The combined filtrates—a green alcoholic solution—were concentrated by distillation *in vacuo* with an argon leak on a water bath. The drying was completed with a methyl alcohol drip, and the dry material was extracted with freshly distilled water-free chloroform. The chloroform filtrate was evaporated to dryness and kept at −5° C under argon in flasks closed with well-greased stoppers. One gram plant tissue yielded about 2 mg chloroform

soluble material. For intraperitoneal injection into guinea-pigs the dried chloroform soluble material was either dissolved in isopropyl myristate or taken up in 0.9% sodium chloride solution. Two to three millilitres of isopropyl myristate was used for dissolving the material to be injected into one guinea-pig. When taken up in 0.9% sodium chloride solution the material to be injected into one guinea-pig was taken up in 7 ml. and filtered: the resultant filtrate, amounting to between 3 and 5 ml., was injected.

## RESULTS

### *Sensitivity of guinea-pigs to a 0.6% histamine aerosol*

In the course of about 2 years, 330 control guinea-pigs were exposed to the 0.6% histamine aerosol. Fig. 3 shows the individual variations in survival time. The ordinate gives the number of animals and the abscissa gives the time in min. Each rectangle represents one guinea-pig. If respiration ceased between the first and second min, the position of the rectangle is between 1 and 2, if between the second and third min it is between 2 and 3, and so on. If the animal survived the 20 min exposure, the position of the rectangle is after 20.

In nearly half the animals (46%) respiration ceased during the first 6 min and in over two-thirds (72%) during the first 8 min. Only 13% survived longer than 11 min and 3.6% survived the 20 min period of exposure. These percentages are given in column I of Table 1.

TABLE 1

PERCENTAGE OF GUINEA-PIGS IN WHICH RESPIRATION CEASED DURING THE FIRST 6 MIN (I) AND DURING THE FIRST 8 MIN (II), AND WHICH CONTINUED BREATHING FOR MORE THAN 11 MIN (III) OR FOR THE WHOLE 20 MIN PERIOD (IV) OF EXPOSURE TO A 0.6% HISTAMINE AEROSOL

| No. of animals | Weight of animals in grams | Percentage of animals | | | |
|---|---|---|---|---|---|
| | | I | II | III | IV |
| 330 | | 46 | 72 | 13 | 3·6 |
| 75 | 165–210 | 36 | 64 | 23 | 6·7 |
| 149 | 215–255 | 42 | 68 | 13 | 2·7 |
| 76 | 260–390 | 50 | 75 | 9 | 2·6 |

Seasonal variations in the sensitivity of the guinea-pig were not apparent when the results obtained in different months were compared with each other, but there was a difference dependent on weight. This is shown in Table 1, where 300 of the 330 guinea-pigs shown in the first line (30 not having been weighed) are subdivided into three groups.

The percentage of the 75 guinea-pigs weighing between 165 and 210 g that stopped breathing during the first 6 and 8 min of the exposure to the histamine aerosol is smaller than the percentage of the 76 guinea-pigs weighing between 260 and 390 g. On the other hand, the percentage of the lighter animals which survived longer than 11 and 20 min is greater than that of the heavier ones. The values obtained with the 149 animals weighing between 215 and 255 g lie between those of the other two groups.

Forty-six guinea-pigs were re-exposed to the histamine aerosol between 24 hr and 7 days after the first exposure. The difference in survival time between the two

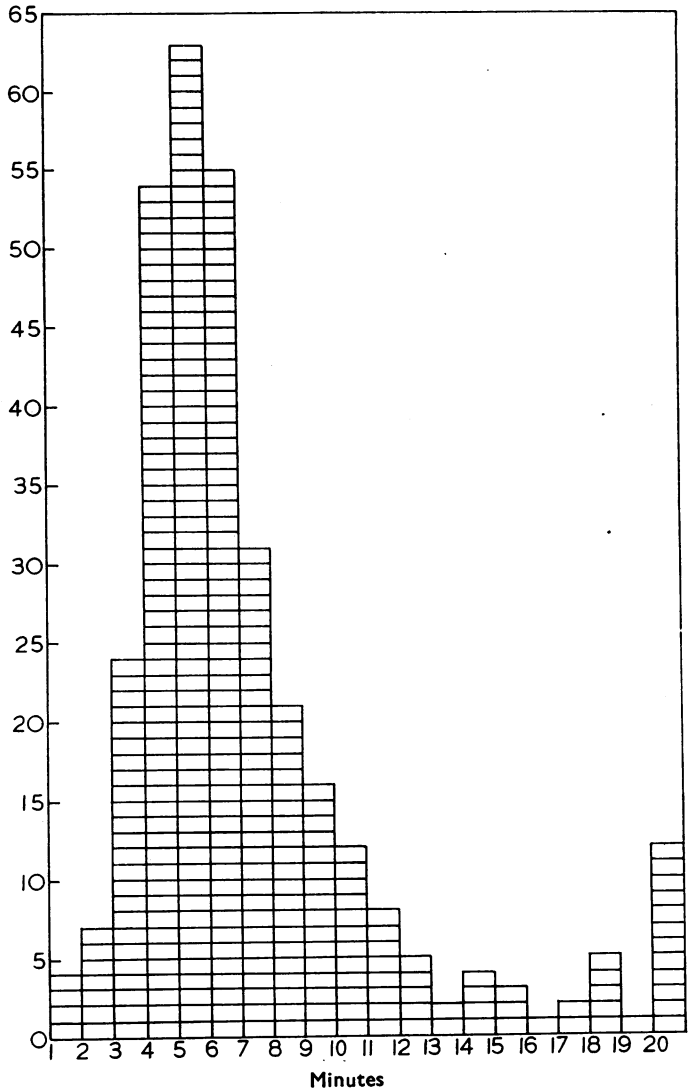


Fig. 3. Variations in sensitivity of control guinea-pigs exposed to a 0.6% histamine aerosol. Each square refers to a single pig. The abscissa is time in minutes from the beginning of the aerosol and the position of the square gives the minute during which the animal's respiration ceased. If the animal survived the 20 min exposure, the square is given beyond 20. Ordinate: number of animals.

exposures is shown in Fig. 4. In 24 of the guinea-pigs, breathing stopped later during the first and in 12 during the second exposure. In 9 the difference was less than 30 sec. These results show that there is a tendency for the guinea-pigs to become less resistant to the histamine aerosol on re-exposure. This is particularly striking for the 8 guinea-pigs which survived the first exposure for the 20 min period, because only two did so during the second exposure, the survival times of the

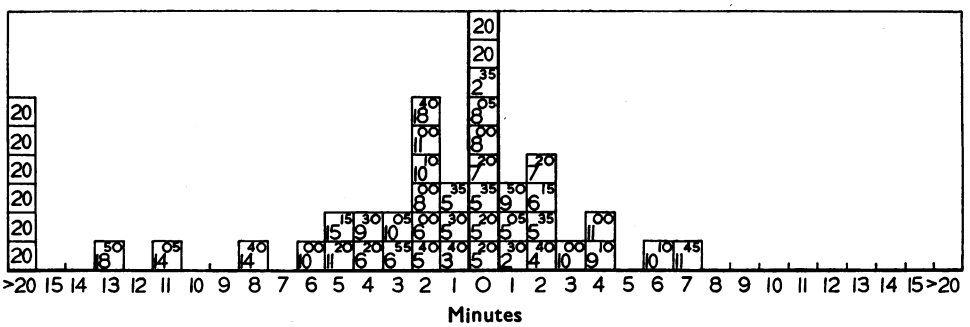


Fig. 4. Difference in survival time of guinea-pigs between a first and second exposure to a 0.6% histamine aerosol. The abscissa gives this difference in minutes on the left of 0, if the survival time was longer during the first, and on the right if longer during the second exposure. The numbers in the squares represent the survival times of the first (on the left) or the second (on the right) exposure, or the mean survival time between the two if the difference was less than 30 sec (above 0). The numbers 20 in the squares above 0 mean that both animals survived the 20 min exposure.

other six animals being 4 min 50 sec ; 6 min 10 sec ; 6 min 35 sec ; 6 min 40 sec ; 9 min ; and 10 min 45 sec.

Fourteen guinea-pigs were re-exposed to the histamine aerosol more than twice within 22 days. As shown in Table 2, different survival times were obtained for the same animal. None of the guinea-pigs which survived the first exposure 11 min or less, however, survived longer than 10 min in any of the subsequent exposures. There was no indication that the animals became more resistant to the histamine aerosol on repeated exposure.

TABLE 2

SURVIVAL TIMES IN MINUTES OF 14 CONTROL GUINEA-PIGS ON REPEATED EXPOSURES TO A 0·6% HISTAMINE AEROSOL AT INTERVALS OF FROM 1 TO 9 DAYS

| | Number of guinea-pig | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Days | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 |
| 0 | $5^{10}$ | $5^{25}$ | $5^{40}$ | $7^{30}$ | $8^{00}$ | $8^{55}$ | $10^{00}$ | $10^{30}$ | $11^{00}$ | $15^{15}$ | 20 | 20 | 20 | 20 |
| 1 | | | $3^{25}$ | | | | | | | $10^{10}$ | | | | |
| 2 | $5^{35}$ | $5^{10}$ | | $7^{15}$ | | | | | | $5^{40}$ | $4^{50}$ | $6^{10}$ | $6^{35}$ | |
| 3 | | | | | | | $3^{50}$ | $8^{10}$ | | | | | | |
| 4 | | $3^{45}$ | $6^{45}$ | | | | | | | | | | | 20 |
| 5 | $6^{30}$ | | | | $8^{10}$ | $9^{50}$ | | | | | | | | |
| 6 | | | | $8^{40}$ | | | | $9^{30}$ | | $10^{05}$ | | $11^{20}$ | | |
| 7 | | $4^{40}$ | $9^{45}$ | | | | | | | 20 | | | | |
| 8 | | | | | | $3^{30}$ | $6^{40}$ | | | | | | | |
| 9 | $7^{40}$ | | | | $9^{30}$ | | | | | | $4^{05}$ | | $4^{40}$ | |
| 12 | | | | | | | | | | | | | | $11^{30}$ |
| 13 | | | | | | | | | $8^{55}$ | | | | | |
| 15 | | | | | | $5^{15}$ | | | | | | | | |
| 22 | | | | | | | | | $9^{35}$ | | | | | |

*Extracts from oak galls*

*Hungarian oak galls.* An intraperitoneal injection into guinea-pigs of the chloroform soluble material of the chloroform methanol extract produced strong protection against subsequent exposure to the 0.6% histamine aerosol. Seven

different batches of extract were used and 29 animals were injected. The amount of extract injected into each animal corresponded to about 5 g oak gall; but its dry weight varied, in the seven batches, between 6 and 32 mg. The results are shown in Fig. 5A. In 28 experiments the injected animal survived longer than its control; in one experiment the difference was less than 30 sec. Twenty of the injected animals survived the 20 min period of the aerosol; the average survival time of their controls was 6 min.

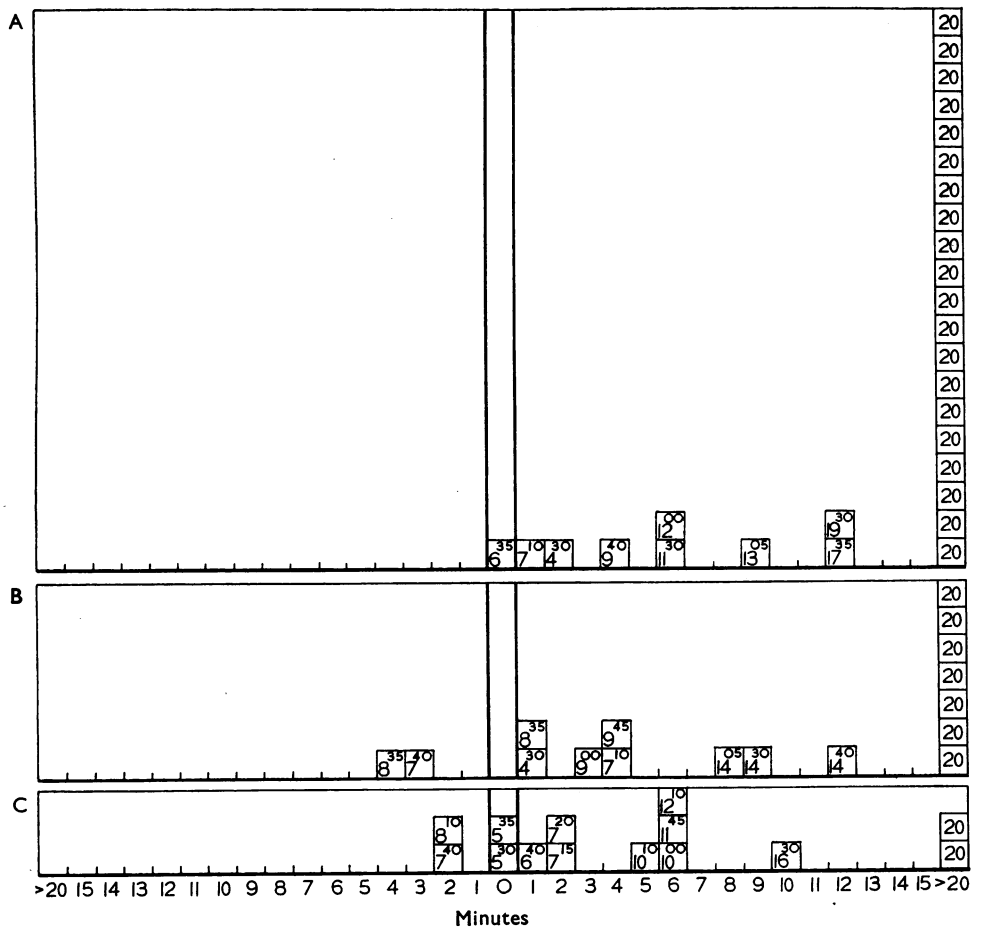


Fig. 5. Protection produced by intraperitoneal injection of extract prepared from Hungarian (A and B) or English (C) oak galls in guinea-pigs exposed to a 0.6% histamine aerosol. At A and C, exposure 4 to 6.5 hr after injection; at B, response 1 to 5 days later. In this and the following figures the abscissa gives, in minutes, the differences in survival time between injected animals and their controls, on the right of 0 if the injected and on the left if the control survived longer, and as 0 if the difference was less than 30 sec or if both animals survived the 20 min exposure. The numbers in the squares on the right of 0 represent the survival times of the injected, and on the left of 0 of the controls, and the numbers in the squares above 0 the mean survival times between the two.

The dried chloroform soluble part of the chloroform methanol extract remained stable when kept under argon at −5° C. In fact, in 13 of the 20 animals that survived the 20 min exposure the extract had been kept in this condition for 4 to 7 days, and in one it had been kept for 32 days.

Seventeen of the injected animals which survived the 20 min period were re-exposed to the histamine aerosol 1 to 5 days later, either together with the revived control from the first test, or with a new control. Fig. 5B shows that 15 of the injected animals survived longer than their controls and that 7 again survived the 20 min period.

Five of these injected animals were re-exposed to the histamine aerosol several times. The results are given in Table 3. Protection appears to last for more than

TABLE 3

SURVIVAL TIMES IN MINUTES OF 4 GUINEA-PIGS INJECTED WITH EXTRACT OF HUNGARIAN OAK GALL AND OF THEIR CONTROLS (NUMBERS IN BRACKETS) ON REPEATED EXPOSURES TO A 0·6% HISTAMINE AEROSOL 1 TO 9 DAYS AFTER THE INJECTION

| | Number of guinea-pig | | | | |
|---|---|---|---|---|---|
| Days | 1 | 2 | 3 | 4 | 5 |
| 0 | 20 (6$^{00}$) | 20 (5$^{40}$) | 20 (11$^{20}$) | 20 (7$^{10}$) | 20 (5$^{10}$) |
| 1 | | 20 (3$^{35}$) | | | |
| 2 | 20 (5$^{10}$) | | 9$^{00}$ (6$^{25}$) | 20 (10$^{30}$) | 20 (5$^{35}$) |
| 4 | 9$^{05}$ (3$^{45}$) | 14$^{45}$ (6$^{45}$) | | | |
| 5 | | | 13$^{50}$ (6$^{45}$) | 19$^{35}$ (8$^{10}$) | 20 (6$^{30}$) |
| 7 | | 20 (9$^{45}$) | | | |
| 8 | | | 12$^{15}$ (9$^{45}$) | | |
| 9 | | | | 20 (9$^{30}$) | 13$^{00}$ (7$^{40}$) |

a week. In experiments 2 and 5 the control animals used for the first exposure were used for subsequent exposures, and in experiment no. 4 the same control animal was used for the second, third and fourth exposures. In all other instances new controls were used for each exposure.

The chloroform soluble material of the chloroform methanol extract prepared from oak galls which were blown due to the escape of the insect, or which had become mouldy, when injected intraperitoneally into guinea-pigs did not produce protection against the histamine aerosol.

*English oak galls.* The chloroform soluble material of chloroform methanol extracts injected intraperitoneally into guinea-pigs produced protection against the histamine aerosol, but the effect was weaker than that produced by extracts prepared from the Hungarian galls.

The results of 14 experiments are shown in Fig. 5C. Each injected animal had been injected intraperitoneally 4 to 6 hr before the exposure to the histamine aerosol, with between 46 and 100 mg dry weight of the chloroform soluble material corresponding to between 5 and 15 g oak gall powder. In ten experiments the injected animal survived longer than its control, and in two it survived the 20 min period. In two experiments the difference in survival time was less than 30 sec and in another two the control animal survived longer.

*Extracts from crown gall tumours of infected tomato plants*

An intraperitoneal injection of the chloroform soluble material obtained from the frozen stems protected guinea-pigs against a subsequent histamine aerosol. No difference was found in the extracts prepared from stems that had been kept frozen for a day or for several months. However, if they had been thawed on various occasions during the storage period the extracts prepared from the stems were less active. The dried chloroform soluble material could be stored at $-5°$ C under argon for several days without loss of activity. There was some difference in activity whether the dried chloroform soluble material used for injection was dissolved in isopropyl myristate or taken up in 0.9% sodium chloride solution.

*Chloroform soluble material dissolved in isopropyl myristate.* Ten to fifteen min after an intraperitoneal injection of extract equivalent to 60 to 75 mg dry weight, there were signs of strong sedation. The animal did not move about and, when left undisturbed in its cage, lay either on its belly or half on its side with its eyes closed. When the animal was placed on its back or on its side, it did not right itself; when placed on its belly, the half-flexed hind legs were not withdrawn under the body and could be fully extended with, at most, only slight resistance from the animal. An extended hind leg was not withdrawn when released. This central effect lasted for several hours. During this time the animals were often definitely colder and the coat was ruffled. Most animals showed severe diarrhoea. After the injection of smaller doses (30 mg dry material) the central effects were less pronounced. After injection of 100 mg dry material or more, several of the injected animals died during the following hours or during the next day.

When the injected animals were exposed 4 to 6 hr after the injection to the 0.6% histamine aerosol they were found to be protected. This is shown in Fig. 6A and B. In the 20 experiments given in A, the amount of dry material injected into each animal was 100 mg in one, 60 to 70 mg in fifteen, and 45 to 50 mg in four. Sixteen of the injected animals survived the 20 min exposure; this happened twice with the controls. The mean survival time of the controls for the 14 experiments in which the injected animals alone survived the 20 min period of exposure was less than 9 min. In none of the 20 experiments did the control survive the injected animal.

In the 7 experiments given in Fig. 6B the amount of dry material injected into each animal was between 25 and 30 mg; the injections still produced protection since all injected animals survived longer than their controls, but the protection was not as strong as that produced by the larger amounts of extract.

The protection was present 1 hr after the injection. In fact, in one of the experiments given in Fig. 6A the interval between injection and exposure to the aerosol was 1 hr. The injected animal survived the 20 min period whereas its control stopped breathing after less than 11 min. Protection was no longer evident 24 hr after the injection or later. Eleven of the injected animals of Fig. 6A which had survived the 20 min period of exposure were re-exposed to the histamine aerosol 1 to 4 days later. As shown in Fig. 6C, protection was no longer evident.

No difference in activity was found with the dried chloroform soluble material whether it was prepared from crown gall tumours alone, or from severely or less

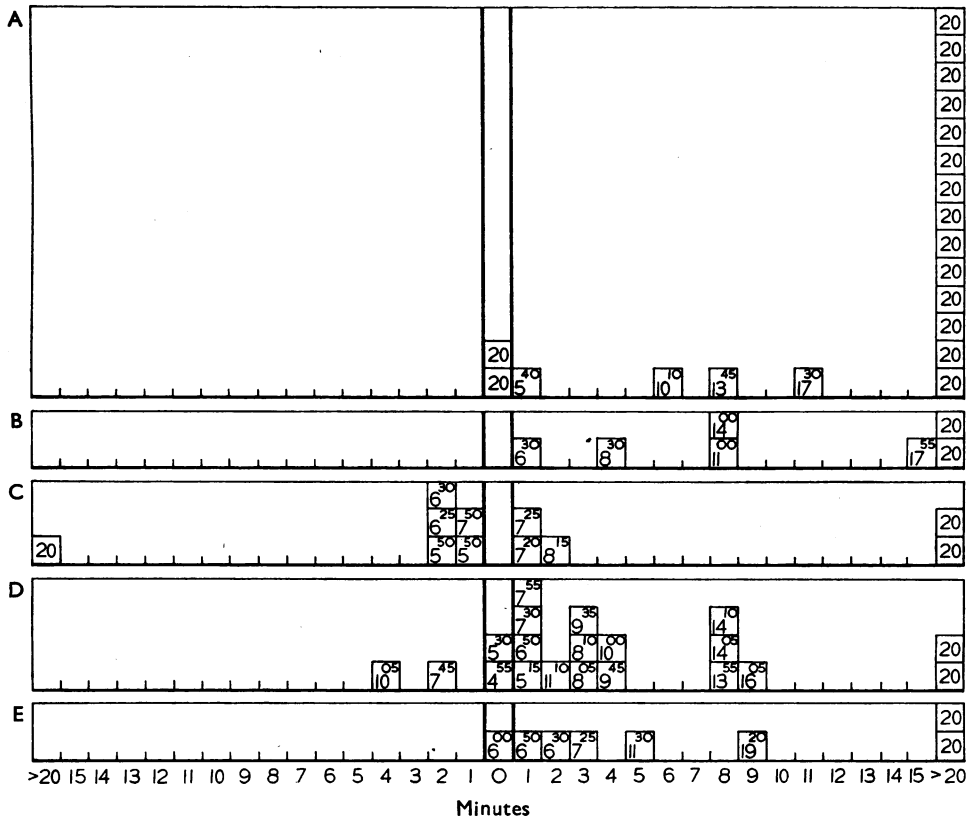


Fig. 6. Protection produced by intraperitoneal injection of extract prepared from crown gall tumours of infected tomato plants (A to D) and from non-infected plants (E) in guinea-pigs exposed to a 0.6% histamine aerosol. Extracts used at D prepared from freeze-dried tissue. All extracts taken up in isopropyl myristate. At A, B, D and E, exposure 4 to 6 hr after injection; at C, re-exposure 1 to 4 days later. Details same as in Fig. 5.

severely infected parts of the stem. The only difference was that the less infected stems yielded nearly 4 times less chloroform soluble dry material per gram fresh tissue than the heavily infected stems or the tumour tissue alone.

Freeze drying of moist tissue reduced the activity since extracts prepared from freeze-dried powder were less active than similarly prepared extracts from frozen stem. The effect produced by extracts from freeze-dried powder is shown in Fig. 6D. Each injected animal had been injected intraperitoneally with 90 to 270 mg dry weight extract equivalent to 5 to 15 g powder and was exposed to the histamine aerosol 4 to 6 hr later. In 16 of the 20 experiments the injected animal survived longer than its control and in 2 it survived the 20 min period ; in 2 the control animal survived longer, and in 2 the difference in survival time was less than 30 sec.

*Chloroform soluble material taken up in 0.9% sodium chloride solution.* Between 110 and 160 mg, usually between 140 and 160 mg, of the dried chloroform soluble

material was used for injection into each animal. Only a small amount was taken up in the 0.9% sodium chloride solution. In one experiment the amount taken up from 50 mg dry material was determined; it was 1.44 mg.

A few minutes after the intraperitoneal injection of the extract, sedative effects were seen similar to but much weaker than those described after the injection of the extracts dissolved in isopropyl myristate. They lasted for several hours. The animals were also colder and their coats were ruffled, but diarrhoea was only occasionally seen. The animals usually lost some weight during the next few days; the loss was greater than that observed in control animals exposed to a histamine aerosol.

The protection exerted by 110 to 160 mg of dry material taken up in 0.9% sodium chloride solution is shown in Fig. 7A. In 22 of the 24 experiments given in Fig. 7A the interval between injection and exposure to the histamine aerosol was

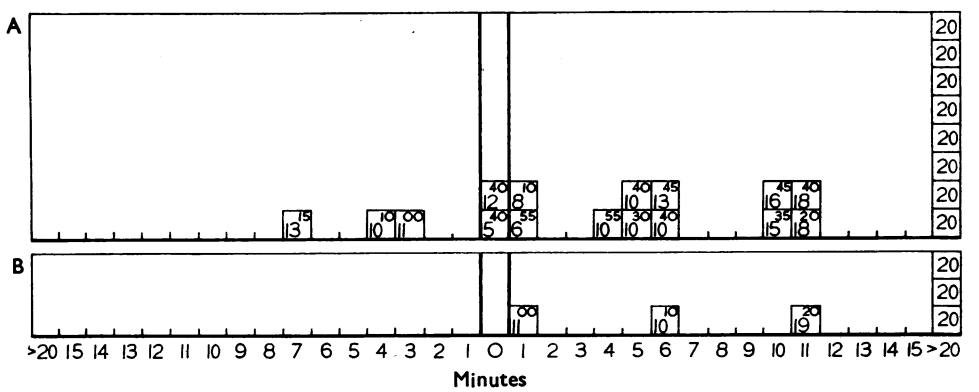


Fig. 7. Protection produced by intraperitoneal injection of extract prepared from crown gall tumours in guinea-pigs exposed to a 0.6% histamine aerosol 1 to 6 hr later (A) and re-exposed 2 to 6 days later (B). Extract taken up in 0.9% sodium chloride solution. Details as in Fig. 5.

4 to 6 hr; in one it was 90 and in another 70 min. In 19 experiments, including the two with the short interval between injection and exposure, the survival time of the injected animal was longer than that of the control, in three the reverse result was obtained, and in two there was no difference.

In the three experiments in which the control survived longer than the injected animal, and in one of the two in which there was no difference in survival time, the extracts were prepared from the last portion of a batch of frozen stems stored in a tin for several months during which time material was periodically taken out. Some deterioration of the active principle in the stems may have occurred, although care was taken to avoid thawing during each removal.

Six of the eight injected animals which survived the 20 min period of the histamine aerosol were re-exposed to the aerosol 2 to 6 days later. All survived longer than their controls and three again survived the 20 min period (Fig. 7B). This result contrasts with that of the corresponding experiments (Fig. 6C) in which the injected extract had been taken up in isopropyl myristate.

TABLE 4

SURVIVAL TIMES IN MINUTES OF 9 GUINEA-PIGS INJECTED WITH THE CHLOROFORM SOLUBLE MATERIAL OF CROWN GALL TUMOUR TAKEN UP IN 0·9% SODIUM CHLORIDE SOLUTION, AND OF THEIR CONTROLS (NUMBERS IN BRACKETS) ON REPEATED EXPOSURES TO A 0·6% HISTAMINE AEROSOL UP TO 47 DAYS AFTER THE INJECTION

| Days | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|---|
| 0 | 20 (7$^{15}$) | 20 (12$^{10}$) | 20 (8$^{20}$) | 20 (5$^{40}$) | | 18$^{30}$ (7$^{55}$) | 16$^{45}$ (6$^{20}$) | 15$^{50}$ (7$^{20}$) | 5$^{45}$ (10$^{10}$) |
| 2 | 20 (20) | | | | | | | | |
| 3 | | 11 (10) | | 10$^{10}$ (4$^{40}$) | | | | | |
| 5 | 9$^{35}$ (9$^{25}$) | | | | | | | | |
| 6 | | | 20 (4$^{40}$) | | | | | | |
| 7 | | | | | 11$^{20}$ (3$^{10}$) | 5$^{05}$ (6$^{20}$) | 8$^{50}$ (10$^{45}$) | | 3$^{20}$ (8$^{40}$) |
| 8 | | | 20 (6$^{35}$) | | | | | | |
| 10 | | 18$^{30}$ (10$^{30}$) | | 20 (5$^{55}$) | | | | | |
| 13 | | | 20 (5$^{15}$) | | 20 (6$^{20}$) | | | 19 (8$^{55}$) | |
| 14 | | | | 20 (5$^{15}$) | | | | | 11$^{10}$ (4$^{35}$) |
| 16 | | | 12$^{40}$ (6$^{35}$) | | | | | | |
| 19 | | 19$^{35}$ (5$^{45}$) | 20 (12$^{20}$) | | | | | | |
| 21 | | | | | | | | | 6$^{15}$ (6$^{50}$) |
| 22 | | | | | 6$^{30}$ (3$^{40}$) | | | 20 (9$^{35}$) | |
| 23 | | | 20 (4$^{40}$) | | | | | | |
| 26 | | 6$^{45}$ (5$^{10}$) | | | | | | | |
| 27 | | | | 20 (12$^{30}$) | | | | | |
| 30 | | | 19 (5$^{55}$) | | 9$^{10}$ (7$^{25}$) | | | 7$^{20}$ (6$^{50}$) | |
| 31 | | | | 20 (11$^{30}$) | | | | | |
| 42 | | | | 20 (5) | | | | | |
| 47 | | | | 13 (5$^{40}$) | | | | | |

Four of the six injected animals were again re-exposed to the histamine aerosol at intervals varying between 2 and 13 days, and in three of these protection was evident for as long as 19, 30 and 47 days. The details of re-exposure of these four experiments are given in the experiments 1 to 4 of Table 4.

The experiments 7 to 11 of this table give the results of repeated exposures of injected guinea-pigs which did not survive the first exposure for the full 20 min period. The extracts used for injection into these animals had been prepared from the last portion of a batch stored in a tin for several months, and as mentioned before, there was a suspicion that some deterioration of the active principle had occurred in the stems during the storage. Some evidence for prolonged protection was obtained in two experiments only (nos. 5 and 8). In one of the two, the first exposure was carried out seven days after the injection. In experiment no. 11 the survival time of the injected animal during the first exposure was shorter than that of its control, and there was also no evidence of delayed protection when re-exposures were carried out during the following three weeks.

*Effect on isolated guinea-pig ileum preparation.* It had been shown that extracts of Hungarian oak gall taken up in isopropyl myristate when added to the bath in which the isolated guinea-pig preparation had been suspended exerted at most only a very weak and irregular antihistamine-like activity (Feldberg & Kovacs, 1960). The possibility was discussed that the failure to obtain the antihistamine effect in these conditions was due to the fact that the active principle was not soluble in watery solutions. In the present experiments no antihistamine-like activity could be obtained on the isolated guinea-pig preparations with the chloroform soluble material of crown galls taken up in 0.9% sodium chloride solution. The addition of extract equivalent to 1 to 2 mg of the chloroform soluble material to the 5 ml. bath did not reduce, but increased, the histamine contraction. Larger amounts equivalent to 5 mg of the material produced a slow contraction. When the extract was washed out and the bath fluid repeatedly changed, it required 15 to 30 min before full relaxation of the muscle had taken place. There was also no evidence of an atropine-like action of the extracts on the guinea-pig ileum preparation.

### *Extracts from stems of non-infected tomato plants*

A few preliminary experiments were made with chloroform soluble material prepared from the frozen stems of non-infected plants. Unfortunately, plants of the same age and size as the infected ones were not available. Some plants were young ones with rather soft stems, others were old plants of another variety from which the ripened tomatoes had been collected. The dried chloroform soluble material was taken up in isopropyl myristate before injection.

The intraperitoneal injection of 100 mg of the dried material prepared from the young plants, equivalent to 125 g fresh tissue, did not protect the guinea-pigs exposed to a 0.6% histamine aerosol 5 hr later. On the other hand, the material prepared from the old stems produced some protection, but to a lesser degree than that produced by the extracts prepared from infected plants as shown in Fig. 6E.

The amount of dried chloroform soluble material injected into each animal varied between 60 and 135 mg, equivalent to 30 to 60 g fresh tissue. In all 8 experiments,

except one, the injected animal survived its control. In the one exception the difference in survival time was less than 30 sec. The two injected animals which survived the 20 min period had each received 135 mg of the dried chloroform soluble material.

## DISCUSSION

The results of the present experiments confirm the previous findings that extracts prepared from plant tumours when injected intraperitoneally into guinea-pigs protect the animals against a lethal histamine aerosol. Potent stable extracts were obtained from two kinds of tumours, from oak galls and from crown galls of infected tomato plants. The active principle obtained from both tumours was soluble in chloroform, and it may well be that the same substance is active in both extracts or that we are dealing with different but related substances.

The most striking result was the long-lasting protection which resulted from a single injection of extract. With oak gall extract protection lasted for several days, with crown gall extract for several weeks. The finding that extracts of crown gall produced this long-lasting protection only when taken up in saline solution and not when taken up in myristate may simply be due to the fact that greater amounts of the active principle have to be injected in order to produce the long-lasting protection. In the experiments with myristate this condition could not be attained because of the toxicity of the extracts. Unfortunately, the supply of oak galls as well as of crown galls was limited so that it was not possible further to investigate this as well as other pertinent problems. For instance, we do not know if we are dealing with a specific antihistamine effect or if the extracts would also protect a guinea-pig against a bronchospasm produced by substances other than histamine. Nor do we know if the increased resistance against histamine is shared by all histamine sensitive smooth muscles of the guinea-pig. Concerning the mode of action by which the protection is produced, we only know that it must be different from that of the synthetic antihistamines, since the saline extracts of the crown gall tumours did not exert an antihistamine effect when added to the bath in which an isolated guinea-pig ileum preparation was suspended. The long-lasting effect suggests an indirect mode of action of the active principle on the smooth muscles, similar to the alleviating effect on allergic reactions of cortisone and the adrenocorticotrophic hormone via the suprarenal cortex.

Apart from protecting the animals against a histamine aerosol the crown gall extracts produced central actions and severe diarrhoea, and when too large an amount of extract was injected the animal died. As these effects were much milder with extracts taken up in saline solution instead of in myristate, they appear to be due to a different substance from that which brings about the protection against the histamine aerosol.

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