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ANTIHISTAMINE ACTIVITY OF EXTRACTS PREPARED FROM BUFFY-COAT LAYER OF HORSE BLOOD AND FROM OAK GALL

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It has been shown that extracts prepared from leucocytes (Kovacs, 1950; Kovacs & Juhasz, 1951, 1952) and from oak gall (Kovacs & Szabadi, 1950) injected into a guinea-pig, protect the animal against a lethal histamine aerosol. The present experiments confirm these results. In addition, it was found that the injection of extracts of the buffy-coat layer of horse blood reduced the sensitivity of the guinea-pig ileum to histamine. Further, in a guinea-pig protected by oak-gall extract, the histamine aerosol produced characteristic behavioural changes which are attributed to a central action of histamine. The protective action of oak-gall extract against the histamine aerosol was recorded in unanaesthetized and in anaesthetized animals. Some of these results have been reported to the Physiological Society (Feldberg & Kovacs, 1959).

METHODS

Testing the sensitivity of the guinea-pig ileum to histamine

In order to test the sensitivity of the guinea-pig ileum to histamine the following standard procedure was adopted. Guinea-pigs weighing 200–300 g were killed by a blow on the head; immediately afterwards a piece of ileum near the caecum was removed and a piece 7–8 cm long was suspended in a 15 ml. bath containing magnesium-free Tyrode solution at 34° C, through which a mixture of 95% O_2 + 5% CO_2 was bubbled. The contractions of the preparation were recorded on a smoked drum by a frontal-writing lever, using the same lever and the same magnification, which was 1:2, throughout. Immediately the preparation was set up, 0·08 μg histamine (base) was added to the bath for 20 sec, and the size of the contraction was recorded. The administration of 0·08 μg histamine was repeated at least ten times at 2 min intervals so that the ileum developed to the full the response of which it is capable at this dose. If the tenth administration caused no or only a small contraction, larger doses of histamine were given.

Testing for bronchoconstriction produced by a histamine aerosol

Two methods were used to study in guinea-pigs weighing 200–300 g the protective effect of oak-gall extracts on the bronchospasm elicited by a histamine aerosol.

In the one method an unanaesthetized guinea-pig was put into a chamber through which a histamine aerosol was passed and the behaviour of the animal was observed through the

glass walls. The chamber was 15 cm wide, 37 cm long and 27 cm high. A 0·6 or 1% histamine diphosphate solution was passed through the chamber as an aerosol with a Collison inhaler at a pressure of 1/4 atmosphere from a compressed air cylinder.

In the other method a guinea-pig was anaesthetized by subcutaneous injection of a 25% solution of urethane, 6 ml./kg. One to two hours later, when the animal was deeply anaesthetized, it was tied on its back in a specially constructed chamber and the respiratory movements of the chest and abdomen were recorded on a smoked drum, as illustrated in the diagram Fig. 1. The movements of the chest and abdomen are transmitted to a lever via a hinged flap made of Perspex. An upward movement of the flap due to an inspiration causes a downward stroke of the lever. A little spring is attached to the free end of the flap so as to reduce the large sudden excursions which might result, during broncho-constriction, from the intense inspiratory efforts with their wide-spread muscular contractions. A Perspex box (22 cm wide, 35 cm long and 10 cm high) was placed over the anaesthetized

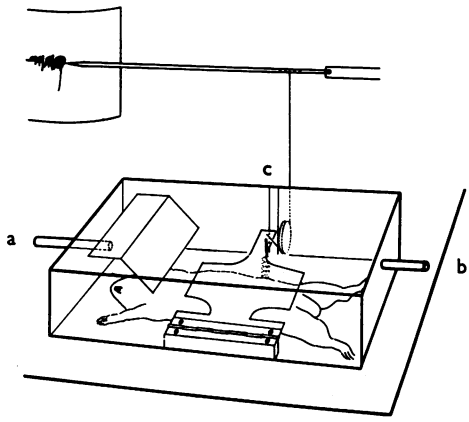

Fig. 1. Diagram of the chamber used to study the effect of a histamine aerosol on the respiratory movements in anaesthetized guinea-pigs. (*a*) Inlet tube for the aerosol. (*b*) Escape tube. (*c*) Narrow slit for the thread connecting the Perspex flap with the lever.

animal and a 1% histamine diphosphate aerosol was passed at a pressure of 1/4 atmosphere from a Collison inhaler through the opening (*a*) and allowed to escape through the opening (*b*) and the narrow slit (*c*).

Preparation of extracts from the buffy coat of horse blood

Crude extracts. Horses which had been immunized, whose plasma was to be used for other purposes, were bled directly from the jugular vein into Winchester bottles containing 10% potassium oxalate (16·5 ml./l. blood). The blood corpuscles were allowed to settle and the plasma was removed.

The corpuscle sediment was kept in the cold for 24 hr–2 weeks before the white layer on top was sucked off and used for preparing the extract. From a batch of 150–200 l. blood about 0·5–1·5 l. of whitish red mush was obtained. The mush was centrifuged and the white layer was sucked off. This procedure was repeated and 300–500 ml. of mush consisting mainly of white cells was obtained.

The mush was frozen during the night at $-5°$ C and allowed to thaw the following morning and then ground for 20–25 min in a mortar with quartz sand acidified with sufficient N-HCl

to give a pH of between 5 and 5·5. By allowing the mixture to stand for a few minutes the mush could be decanted from the sand into a large flask. The sand was repeatedly washed with distilled water and the washings were added to the flask, the final volume being 3–4 l. This mixture was neutralized with N-NaOH to a pH of between 6·9 and 7, and allowed to stand for 10–15 min, during which time a sediment formed which, when centrifuged off, was the crude extract used for injection into guinea-pigs. One millilitre of crude extract corresponded roughly to 2 ml. of the frozen mush or to nearly 1 l. of horse blood.

Ether–chloroform extract. The crude extract was acidified immediately after centrifugation with N-HCl to a pH of 2 and frozen at −5° C. In this condition it could be kept for several weeks apparently without deterioration. For the ether–chloroform extraction the crude extract was thawed, then neutralized with N-NaOH to a pH of 6·8–7·2, and extracted twice with 2–3 times the volume of ether and then twice with 2–3 times the volume of chloroform. The ether and chloroform solutions were pooled and concentrated to dryness on a water-bath between 30 and 40° C. The dry material was kept under nitrogen at −70° C in a small box of CO_2 snow. The yield of dry material from a bath of 150–250 ml. crude extract was 0·5–1·5 g. Before being used for injection the dry material was dissolved in a small quantity of ether, then isopropylmyristate 2 ml. was added to the solution and the ether removed *in vacuo*; the active material remained dissolved in the isopropylmyristate.

Aluminium oxide column chromatography. The ether–chloroform extract was purified by dissolving the dry material in petrol ether and chromatographing it on an aluminium oxide column. The petrol ether had a boiling point of 30–40° C. About 10–12 ml. was used for each gram of dry material and the column was made up of 45–55 g of aluminium oxide suspended in the petrol ether. The height of the column was 12–13 cm and its diameter was 2·5 cm. The procedure of elution which was carried out under nitrogen was as follows:

1. 100 ml. petrol ether (30–40° C).
2. 100 ml. petrol ether (40–60° C).
3. 50 ml. petrol ether (40–60° C) + 50 ml. petrol ether (60–80°).
4. 100 ml. petrol ether (60–80° C).
5. 95 ml. petrol ether (60–80° C) + 5 ml. chloroform.
6. 90 ml. petrol ether (60–80° C) + 10 ml. chloroform.
7. 80 ml. petrol ether (60–80° C) + 20 ml. chloroform.
8. 70 ml. petrol ether (60–80 °C) + 30 ml. chloroform.
9. 60 ml. petrol ether (60–80° C) + 40 ml. chloroform.
10. 50 ml. chloroform + 50 ml. ether.
11. 250 ml. ether.

Each of the eleven fractions was dried separately *in vacuo*, taken up with 5 ml. ether and divided into two portions of 2 and 3 ml. which were then dried *in vacuo* and kept frozen under nitrogen at −70° C until tested. The 2 ml. portion was used to find out if it had a protective effect against a lethal histamine aerosol in an unanaesthetized guinea-pig; and if so the other 3 ml. was tested for its effect on the sensitivity of the ileum to histamine. Immediately before the injections the portions were dissolved in 0·5–2 ml. isopropylmyristate and injected in this form. Control injections of similar amounts of isopropylmyristate were ineffective.

Preparation of alcoholic extract from oak gall

Hungarian oak galls, the tumours produced by the larvae of *Cynips quercus calicis*, were used. The galls had been stored at −5° C for 2 years. For each experiment 4–6 galls, weighing 18–23 g, were ground in a special plant mill; the powder was extracted with 200 ml. ethanol at 65° C for 5 min, cooled, filtered and re-extracted in the same way with 100 ml. ethanol. The combined filtrate, a brownish alcoholic solution which contains the active principle, amounted to between 210 and 240 ml. Two samples, each of 4–6 ml., were immediately evaporated to dryness *in vacuo*. One sample served to determine the dry weight, which was

between 10 and 15 mg/ml. filtrate, so that the alcohol filtrate from 20 g of the original powder contained between 2 and 3 g dry material. The other sample was taken up in 3–5 ml. saline solution, of which usually half was used for injection into a guinea-pig. The amount of dry weight injected was thus between 20 and 45 mg, usually between 30 and 35 mg.

RESULTS

Effect of injections into guinea-pigs of extracts of the buffy coat of horse blood on the histamine sensitivity of the ileum preparation

In preliminary experiments it was found that extracts of the buffy coat of horse blood added to the guinea-pig ileum preparation suspended in magnesium-free Tyrode solution had a weak and irregular depressant effect on histamine contractions. On the other hand, the histamine sensitivity of ileum preparations obtained from guinea-pigs injected with these extracts was lower than that of preparations obtained from untreated control guinea-pigs.

In untreated control guinea-pigs, the sensitivity of the ileum preparation to histamine tested under the standard conditions described showed surprisingly small individual variations. The range of variation is illustrated in Fig. 2, which shows the development of the histamine responses in a sensitive, an insensitive and an intermediate preparation obtained from different guinea-pigs.

Experiment *A* illustrates the development of the responses in a sensitive preparation. The first administration of 0·08 μg histamine produced a strong contraction, and with each subsequent administration the contractions increased until a maximum was reached after the twelfth administration. At this stage half the dose of histamine (0·04 μg) was sufficient to produce a strong contraction.

On the other hand, in experiment *C*, the first two doses of 0·08 μg histamine were ineffective, but even in this insensitive preparation a relatively strong contraction developed after ten administrations of 0·08μg histamine, and maximal sensitivity was reached after about twenty administrations; and at this stage 0·04 μg histamine also produced a contraction. This preparation illustrates the development of histamine responses as it occurs in insensitive preparations encountered in control guinea-pigs. The development of the responses in a preparation of intermediate sensitivity is illustrated in experiment *B*.

Crude extract. When guinea-pigs were killed a few hours after an intraperitoneal injection of 5–7 ml. of crude extract, and the abdomen was opened, it could be seen that the extract was not fully absorbed. The appearance of the intestine varied; sometimes it had a normal appearance, sometimes it had a greyish colour and was fully relaxed, but the appearance bore no relation to the sensitivity of the ileum preparation to histamine.

When an intraperitoneal injection of the crude extract had been effective in protecting the unanaesthetized guinea-pig against a lethal histamine aerosol, it had also resulted in a reduced sensitivity of the ileum preparation to histamine. This result was obtained with extracts prepared from ten batches of buffy-coat layer. With five the reduction in sensitivity of the ileum preparation to histamine was 1000 times or more, with the other five it was between 2 and 20 times. There was also a reduction in sensitivity to acetylcholine but to a lesser degree. Ileum preparations of untreated guinea-pigs responded usually to 0·04 μg acetylcholine with a contraction

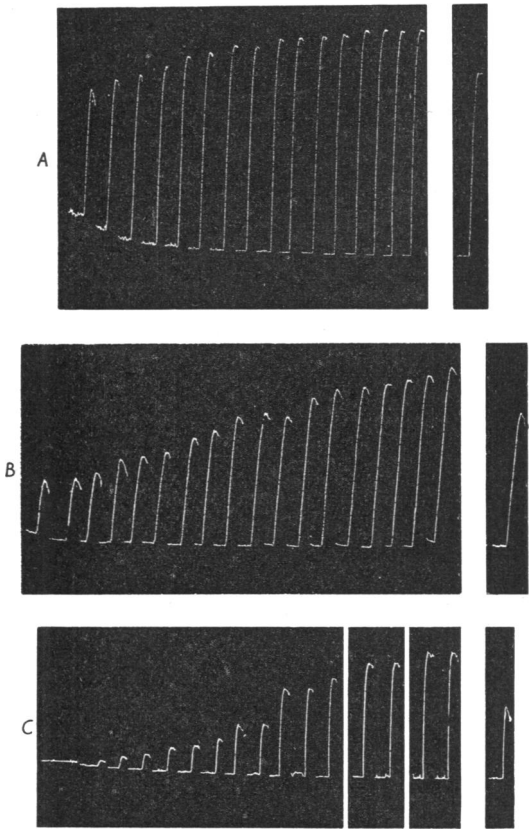

Fig. 2. Development of histamine responses in three ileum preparations from different untreated guinea-pigs. Preparations suspended in 15 ml. magnesium-free Tyrode solution. All contractions except the last ones were in response to 0·08 μg, the last ones to 0·04 μg, histamine. *A*, a sensitive preparation; *B*, a preparation of average sensitivity; and *C*, a relatively insensitive preparation, which did not respond to the first two administrations of 0·08 μg histamine, which are indicated by the white dots. Between each of the two gaps after the first and second blocks at *C* two contractions have been omitted.

equal to that produced by 0·08 μg histamine, the ratio acetylcholine: histamine being about 1:2. In preparations of treated animals, in which the sensitivity to histamine became greatly, and that to acetylcholine slightly reduced, the ratio could become 1:100 or even 1:1000.

The result with a particularly effective extract is illustrated in Fig. 3*A*. The guinea-pig had been injected intraperitoneally with 7 ml. extract and was killed 7 hr later. The ileum preparation from this guinea-pig did not

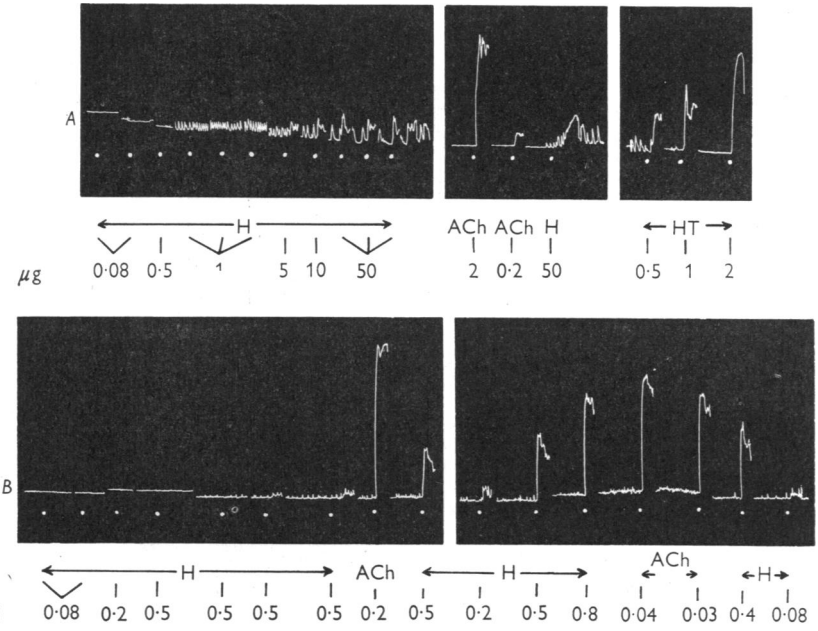

Fig. 3. Development of histamine (H), acetylcholine (ACh) and 5-hydroxytryptamine (HT) responses in ileum preparation, suspended in 15 ml. magnesium-free tyrode solution, from guinea-pigs killed 7 hr after (*A*) an intraperitoneal and (*B*) a subcutaneous injection of 7 ml. crude extract from buffy coat of horse blood: numbers indicate doses. For details see text.

respond to 0·08 μg histamine. In Fig. 3*A* only the last two of ten administrations of 0·08 μg are shown. When the dose of histamine was increased to 50 μg still no contraction ensued. This experiment illustrates also that the sensitivity of the ileum preparation to acetylcholine was reduced. It responded to 0·2 μg acetylcholine with a small, and to 2 μg with a strong, contraction, at a time when it was nearly insensitive to 50 μg histamine. The sensitivity to 5-hydroxytryptamine (5-HT) was reduced as well, but the preparation responded to 0·5 μg 5-HT. Since preparations from untreated guinea-pigs are less sensitive to 5-HT than to histamine, the reduction in 5-HT sensitivity was less than that of acetylcholine.

When the same extract had been injected subcutaneously the reduction in sensitivity of the ileum preparation was less pronounced than after intraperitoneal injection, as is shown in Fig. 3*B*. This ileum preparation again was insensitive to 0·08 μg histamine. The record begins with the last two of ten administrations. At this time the preparation was also insensitive to 0·2 and 0·5 μg histamine, but later it responded to 0·2 with a slight, and to 0·8 μg histamine with a relatively strong response. The sensitivity to acetylcholine was little if at all reduced, since the preparation responded to 0·03 μg acetylcholine with a contraction equal to that produced by 0·8 μg histamine.

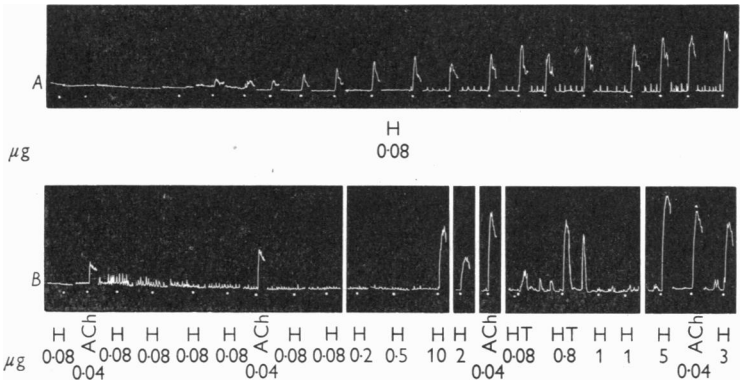

Fig. 4. Development of histamine (H), acetylcholine (ACh) and 5-hydroxytryptamine (HT) responses in ileum preparations, suspended in 15 ml. magnesium-free tyrode solution, from guinea-pigs killed 6 hr after a subcutaneous injection (*A*) of 2·5 ml. and (*B*) of 5 ml. crude extract from buffy coat of horse blood: numbers indicate doses. For details see text.

The degree of reduction in sensitivity depends on the amount of crude extract injected intraperitoneally or subcutaneously. This is shown for subcutaneous injections in Fig. 4. The extract was from a different batch of horse blood from that used in experiment of Fig. 3. The preparation from a guinea-pig injected with 2·5 ml. extract was only a little less sensitive to histamine than an insensitive preparation from a control guinea-pig, since with repeated administration of 0·08 μg histamine contractions were obtained (Fig. 4*A*). On the other hand, a strong reduction in sensitivity was obtained in the preparation from a guinea-pig injected with double the amount of extract (Fig. 4*B*). The sensitivity to acetylcholine was apparently unchanged. When full sensitivity had developed, 0·04 μg acetylcholine gave a contraction stronger than that of 3, and weaker than that of 5 μg histamine, so that the ratio acetylcholine:histamine was about 1:100. There was apparently no decrease in sensitivity to 5-HT,

since the preparation responded to 0·8 μg 5-HT with a strong, and to 0·08 μg with a weak, contraction.

Ether-chloroform extract. Not all ether–chloroform extracts prepared from active crude extract were effective, on intraperitoneal injection, in giving protection against a lethal histamine aerosol or in reducing the sensitivity of the ileum preparation to histamine. Further, when an active ether–chloroform extract was kept, even at $-70°$ C, for a few days, it usually became less active. This instability of the active principle in the extract may well explain why some extracts were inactive from the beginning.

Some ileum preparations from guinea-pigs to which about 60 mg of ether–chloroform extract had been injected 20 hr before the animals were killed contracted only to 20 or 50 μg histamine. The sensitivity to acetylcholine was also reduced, but to a lesser extent. A dose of 0·2 μg acetylcholine was always effective and in one experiment the strong reduction

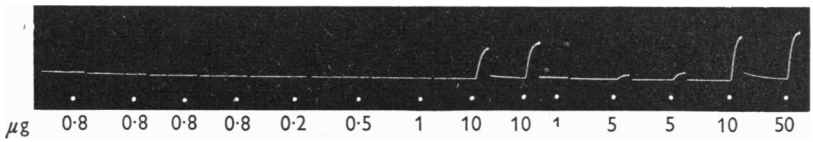

Fig. 5. Development of histamine responses in an ileum preparation, suspended in 15 ml. magnesium-free tyrode solution, from a guinea-pig killed 20 hr after an intraperitoneal injection of 1 mg dry weight of fraction 8 obtained on elution of an ether–chloroform extract from buffy coat of horse blood after chromatography on an aluminium oxide column: numbers indicate doses. For details see text.

in sensitivity to histamine occurred without an apparent change in sensitivity to acetylcholine. A dose of 20 μg histamine produced a contraction equal to that of 0·02 μg acetylcholine, so that the acetylcholine:histamine ratio was 1:1000.

Aluminium oxide column chromatography. Of the 11 fractions obtained nos. 2, 3, 4, 5, 6 and 11 did not show a protective effect against a lethal histamine aerosol when injected intraperitoneally into a guinea-pig, but with one or more of the fractions 1, 8, 9 and 10 protection was obtained in six experiments. Yet in only two of these did extract (1 mg) produce also a definite reduction in sensitivity of the ileum preparation. One of these experiments is illustrated in Fig. 5. The ileum responded to 5 μg with a just perceptible contraction, and the responses to 10 and 50 μg were weaker than those obtained with 0·08 μg in control preparations.

Since a definite reduction in sensitivity was obtained in two experiments only, the failure to obtain this effect in the other four experiments was thought to have been due to the fact that the amount of extract injected with each fraction was too small. Therefore, in one experiment three

effective fractions from three different batches of horse-blood buffy coat were pooled. The effective fractions from the first and second batches were stored at −70° C until the third effective fraction was obtained. The time of storage was a fortnight for the fraction of the first, and a week for that of the second batch. The dry weight of the three fractions was 2·5 mg. When this was injected intraperitoneally, the ileum preparation of the guinea-pig killed 24 hr later did not respond to 100 μg histamine.

Effect of a histamine aerosol on guinea-pigs injected with oak-gall extracts

Unanaesthetized guinea-pigs. The observations were confirmed which had been made by Kovacs & Szabadi (1950) on the protective action of an intraperitoneal injection of an alcoholic extract obtained from oak gall against the lethal effect of a histamine aerosol in unanaesthetized guinea-pigs. The typical effect of a lethal histamine aerosol on an untreated normal guinea-pig is as follows. A few seconds after the beginning of the dispersion of a 0·6–1 % histamine aerosol into the chamber which houses the guinea-pig, its respiration accelerates. A little later the respiration becomes laboured with apparently unsuccessful inspiratory efforts and there is intense gasping. The animal quickly becomes cyanotic and has a frightened aspect. Next it becomes unsteady, swaying from side to side, and rears up suddenly when making an effort to get air into its lungs. It then falls on its side or back, and within a few seconds lethal general convulsions develop which last a few seconds. After cessation of the convulsions there is often urination and sometimes superficial movements of the thorax and abdomen occur and continue for a short time before the animal dies. An untreated guinea-pig usually does not survive the aerosol for more than a few minutes.

A guinea-pig protected aginst a lethal histamine aerosol by an intraperitoneal injection of an alcoholic extract of oak gall (dry weight 20–45 mg) survives the aerosol for over 20 min and sometimes for over 60 min, and when it dies death is not from bronchospasm but apparently from a central action of histamine. The effects of 43 freshly prepared extracts were examined. On intraperitoneal injection a strong protection, i.e. a survival time of at least 20 min during exposure to the histamine aerosol, was obtained with 26 extracts; a less pronounced protection with a survival time of about 10 min was obtained with 10 extracts, and with 7 extracts there were no definite signs of protection, i.e. no prolongation of survival time.

During the survival time the protected animal shows characteristic changes in behaviour. The typical effect of a 0·6–1 % histamine aerosol in a fully protected animal is as follows. Acceleration of respiration, as observed in the unprotected animal, usually occurs and continues for some

time, but there are no signs of unsuccessful inspiratory efforts. At the time when an unprotected animal would be entering the stage of lethal convulsions, the protected animal shows no signs of any further histamine effects. Later, after about 3–5 min, the respiration becomes slower and deeper, and is interrupted from time to time by a deep inspiration, without apparent bronchoconstriction, followed by a period of apnoea. Without rearing or convulsion the animal may fall on its side or take up a crouching position with its head flexed. The deep inspirations followed by short periods of apnoea become more frequent and dominate the pattern of respiration. When at this stage the animal is taken out of the chamber it can be put on its back and makes little or no effort to right itself. And when placed on its belly, the hind legs are not drawn under the body but remain sticking out in a half-flexed position resembling that usually taken up by frogs. This is the result of definite weakness of the legs, particularly the hind legs, which can be flexed and extended without much resistance. The tendon reflexes are present and when the hind paw is pinched the animal withdraws its leg and tries to move away, but the reaction is delayed and more sluggish than in a normal animal. The appearance is that of an animal slightly anaesthetized. When the animals are kept in the aerosol they do not die from bronchospasm, but the respiration becomes slower and deeper, and finally ceases after a few superficial ineffective respiratory movements.

The same difference between the unprotected and the protected un-anaesthetized animal was observed when a lethal dose (histamine base 0·4 mg/kg) was injected into the vein on the back of the thigh. The unprotected animal died within a few minutes, whereas the protected animal showed the characteristic central effects of histamine, a condition of weakness or partial paralysis of the hind legs, light anaesthesia and slow respiration. The protected animal tolerated 1 mg/kg of histamine or more.

Anaesthetized guinea-pigs. The differences in the respiratory response to a histamine aerosol is also observed in guinea-pigs anaesthetized with urethane. Figure 6 shows the typical lethal effect of a 1% histamine aerosol on respiration in three anaesthetized untreated guinea-pigs. The two animals from which the upper records were taken died within 3 min from intense bronchospasm, the last gasp being shown in both records. The sequence of respiratory changes was usually as follows. Within 20–30 sec of the beginning of exposure to the histamine aerosol the animal would take a deep breath and then there would be a short period of superficial polypnoea indicated in the records by a gradual upward movement of the lever, without showing the individual respiratory movements. The respiratory movements then became deeper and there followed a period of laboured respiration with inspiratory dyspnoea. Each forced inspiration gave a

downward stroke on the record; some of the inspiratory efforts were so intense that they involved almost the whole body musculature. In the records they produced the very large downward strokes. Very quickly the forced respiratory movements became less frequent, periods of apnoea occurred which lengthened; from time to time they were interrupted by intense inspiratory efforts involving the whole body musculature. Sometimes, after respiration had stopped for 30 sec or more, a few ineffective respiratory movements reappeared; this happened also after the aerosol

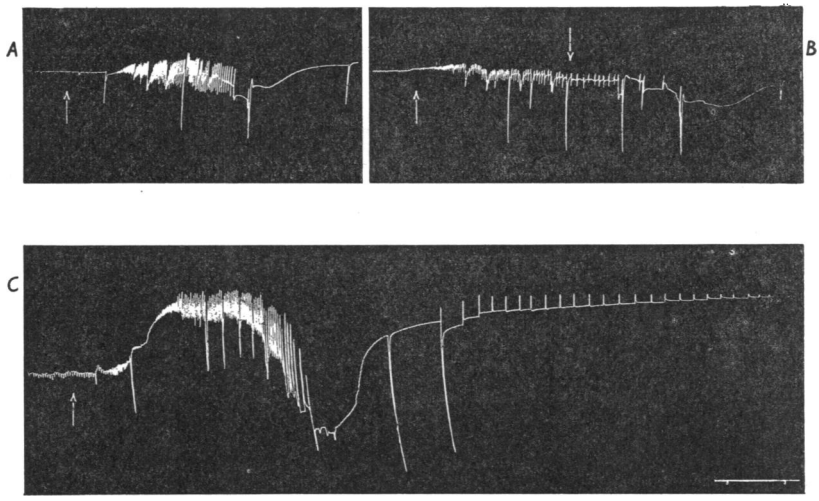

Fig. 6. Records of respiration in three guinea-pigs anaesthetized with urethane; lethal effect of a 1 % histamine aerosol. Beginning of exposure to aerosol indicated by arrow (↑). In guinea-pig *B*, the aerosol was discontinued after 75 sec at second arrow (↓). Time marker, 30 sec. For details see text.

had been discontinued, but they did not bring air into the lung and did not affect the lethal outcome. In the experiment of Fig. 6*C* there was a period of about 3 min of ineffective, gradually decreasing respiratory movements which occurred every 7–8 sec.

Between 30 and 35 mg dry weight of an alcoholic extract of oak gall was required to produce the protection on intraperitoneal injection against the lethal histamine aerosol. Protection occurred within 1 hr and lasted for 24 hr, or sometimes as long as 4 days. In the experiment of Fig. 7 the animal was tested 4 hr and again 4 days after the intraperitoneal injection of the oak-gall extract. Four hours after the injection no signs of bronchoconstriction were produced by the histamine aerosol, but deep inspirations interrupted the normal breathing and became more frequent as the aerosol continued. As shown in the later records (20 and 25 min) the respiration between the deep inspirations became progressively slower and finally

(after 30 min) there was no longer a distinction between deep inspiration and normal respiration, which had become slow, about one respiration every 3 sec. When retested 4 days later (lower record Fig. 7*B*) the effect of the histamine aerosol had scarcely changed; however, when retested 5 days later (not shown in the figure) the histamine aerosol caused intense bronchoconstriction and the animal died within 5 min.

The experiment of Fig. 8 illustrates in another animal the protection attained 1 hr after an intraperitoneal injection of the oak gall extract. In this experiment the histamine aerosol was discontinued after 30 min (at the arrow in *e*). Respiration soon quickened and the deep inspirations became less frequent and ceased within 7 min.

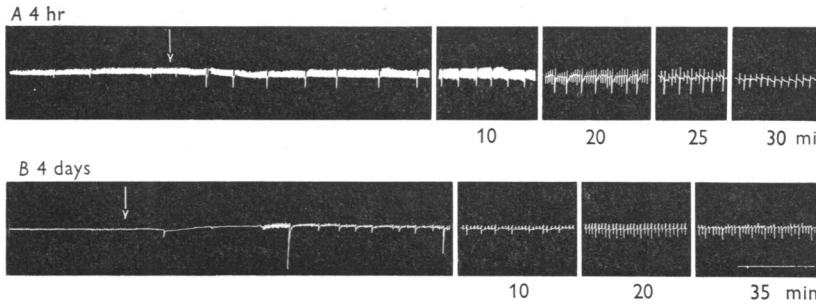

Fig. 7. Records of respiration from a guinea-pig under urethane anaesthesia (*A*) 4 hr and (*B*) 4 days after an intraperitoneal injection of an alcoholic extract of oak gall. At the arrows (↓) beginning of exposure to 1 % histamine aerosol, which was continued until the end of the records; the figures below the records show the time in minutes after the beginning of exposure to the histamine aerosol. Time marker, 30 sec. For details see text.

When only about half the amount of alcoholic extract, corresponding to 15 mg dry weight, was injected intraperitoneally, only slight protection was attained.

The alcoholic extract of oak gall was also effective when injected subcutaneously, but the effect was not so pronounced as after intraperitoneal injection. A typical experiment is illustrated in Fig. 9 in which the histamine aerosol was tested $3\frac{1}{2}$ hr after a subcutaneous injection of an alcoholic extract of oak gall (dry material 35 mg). The extract was from the same batch as that tested in experiment Fig. 8. The animal withstood the histamine aerosol which was continued for 30 min, but at the beginning there was one strong inspiratory effort, as indicated by the large downward stroke which indicated some bronchoconstriction. When the animal was retested 24 hr later, the protection against the histamine aerosol had practically disappeared.

The protective action of the alcoholic extract of oak gall does not result

from the tannic acid present in the extract, because tannic acid injected intraperitoneally into a guinea-pig did not protect the animal, whereas an alcoholic extract of oak gall, from which the tannic acid had been removed by lead hydroxide precipitation, produced protection. The experiment of Fig. 10 shows the protection against a histamine aerosol tested 5 hr after an intraperitoneal injection of about 10 mg tannin-free dry material.

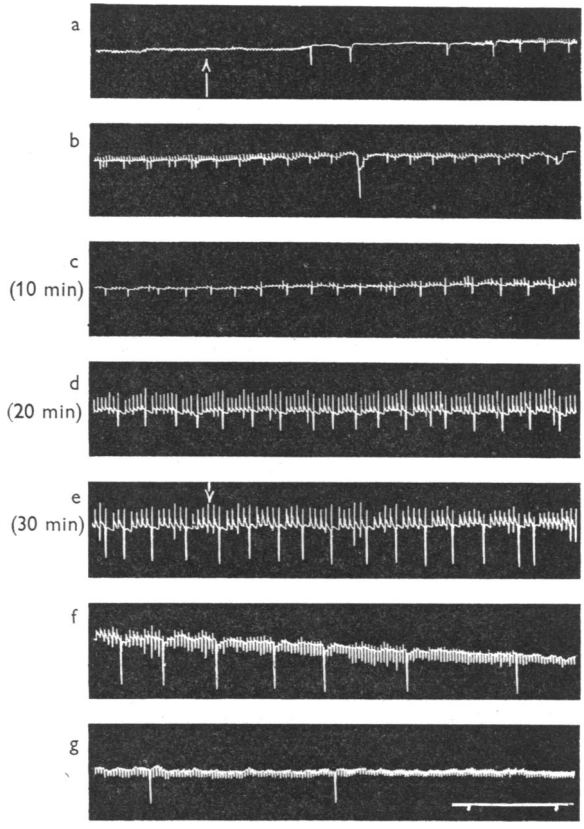

Fig. 8. Records of respiration from a guinea-pig under urethane anaesthesia 1 hr after an intraperitoneal injection of an alcoholic extract of oak gall (dry weight 35 mg). At the arrow (↑) at *a*, beginning of a 30 min exposure to 1 % histamine aerosol which was discontinued at the arrow (↓) at *e*. Tracing *a* and *b*, continuous record; *c*, 10 min, *d* 20 min and *e* 30 min after beginning of histamine aerosol; tracing *e*, *f* and *g*, continuous. Time marker, 30 sec. For details see text.

DISCUSSION

The finding that extracts of the buffy coat of horse blood injected into a guinea-pig selectively lower the sensitivity to histamine, not only of the smooth muscles of the bronchi but also of the intestine, suggests that the

active principle of the extracts affects smooth muscle in general. The present experiments do not deal with the problem of the origin of the active principle in the buffy-coat layer. When Kovacs (1950; Kovacs & Juhasz, 1951, 1952) examined the antihistamine effect of such extracts on the bronchi in guinea-pigs *in vivo*, he found a definite correlation between potency and eosinophil count of the extracts, and suggested that the active principle was derived from the eosinophils. This suggestion is supported by the observation that extracts prepared from isolated eosinophil granules, obtained by digestion of leucocytes with trypsin, exert an antihistamine effect on the isolated guinea-pig ileum (Vercauteren & Peeters, 1952; Vercauteren 1953). Recently, Archer, (1956, 1959)

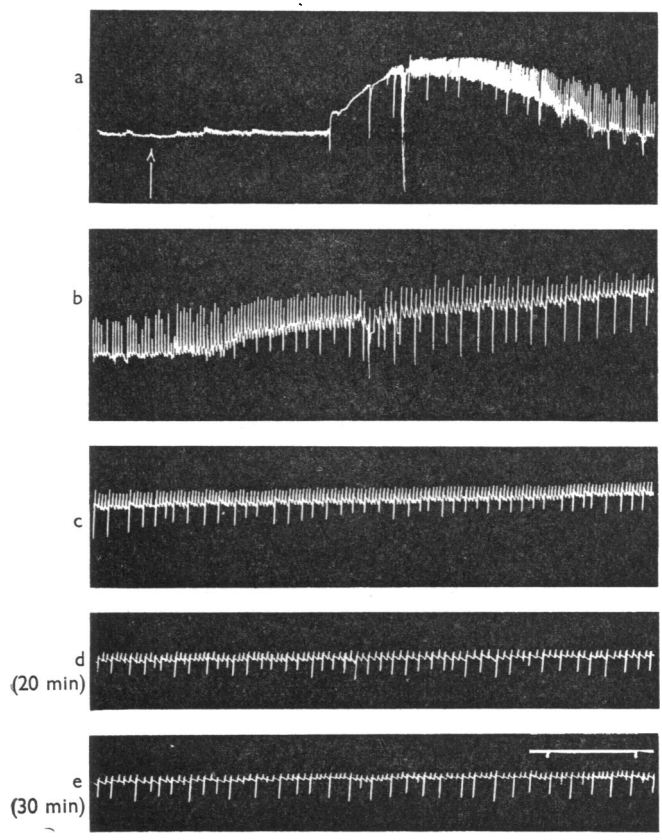

Fig. 9. Record of respiration from a guinea-pig under urethane anaesthesia, 3½ hr after a subcutaneous injection of an alcoholic extract of oak gall (dry weight 35 mg). At the arrow (↑), beginning of exposure to a 1% histamine aerosol which was continued until the end of the record. Tracings *a*, *b* and *c*, continuous record; *d* 20 min and *e* 30 min after the beginning of the histamine aerosol. Time marker, 30 sec. For details see text.

attributed antihistaminic properties to the eosinophils. According to his view the eosinophil leucocytes have a chemotactic affinity to histamine, since he found that injections of histamine into the bone marrow or into the skin of horses produce a local accumulation of eosinophils. Their antihistaminic property was based on the finding that the local oedema normally produced by an intradermal injection of histamine was reduced or abolished in the eosinophil-rich areas. If the active principle of our extracts prepared from the buffy-coat layer were derived from the eosinophils it would not be surprising that the amount of extract required to

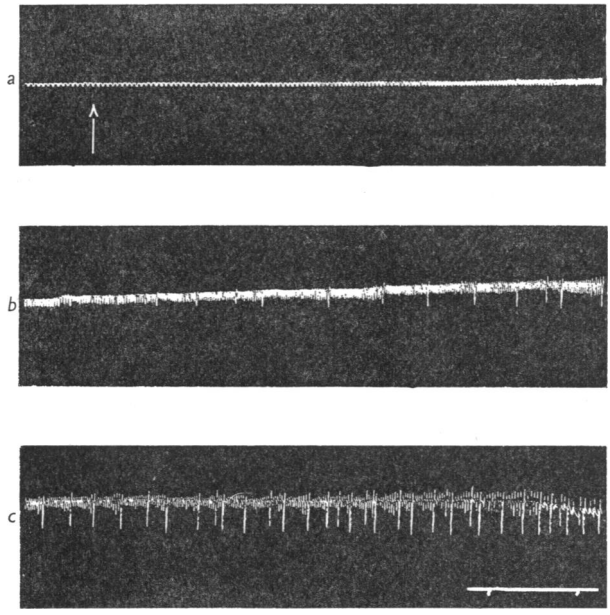

Fig. 10. Record of respiration from a guinea-pig under urethane anaesthesia, 5 hr after an intraperitoneal injection of tannic-acid-free extract of oak gall (dry weight 10 mg). At the arrow (↑) beginning of exposure to 1 % histamine aerosol, which was continued to the end of the record. Tracings *a*, *b* and *c*, continuous record. Time marker, 30 sec. For details see text.

produce the antihistaminic effect corresponds to a relatively large amount of the buffy coat, since it is known that on standing and sedimentation of blood the eosinophils have a tendency to pass into the layer of red cells (R. K. Archer, personal communication).

The fact that the extract of the buffy coat layer had no, or only a weak, antihistaminic effect *in vitro* when added to the bath in which the ileum preparation was suspended can be explained in either way. The active principle may be water-insoluble and therefore not effective when added to the bath fluid, or the effect may not be a direct one on the smooth

muscles, as that of the synthetic antihistamine, but may develop only *in vivo* by an as yet undefined mechanism. It is natural to think in this connexion of the protective action which cortisone and adrenocorticotrophic hormone exert on allergic and inflammatory reactions.

One of the striking features of the antihistaminic action of the alcoholic extracts of oak gall was its long duration. The variability in the effectiveness of our different batches of oak-gall extracts is in agreement with previous observations (Kovacs, Kovacs, Szabady & Varsanyi, 1952). Since the active principle in oak galls seems to be inactivated by oxygen, such variability is more likely to be found when, as in the present experiment, old stored galls are used for extraction. This is because in spring the wasps escape from the galls, leaving fine holes in the skin through which air can enter.

When a guinea-pig was protected against a lethal histamine aerosol by an injection of oak-gall extract the histamine aerosol was not found to be ineffective, but produced changes in respiration and behaviour which were attributed to central actions of the histamine. The guinea-pigs appeared to be partly paralysed or in a condition of light anaesthesia. In other species histamine is known to produce such effects as a result of its central action (for references see Feldberg & Schilf, 1930).

In the untreated guinea-pigs such effects have hitherto not been observed because of the lethal bronchospasm which supervenes so quickly. By protecting the guinea-pigs against this bronchospasm these central effects are unmasked. This does not necessarily mean that the oak-gall extracts have no protective action against the central effects of histamine, it need only be a sign that the effects of histamine on the central nervous system are more resistant than those on the bronchial muscles.

From the experiments performed so far it is not possible to draw conclusions about the chemical nature of the active substance in oak-gall extracts or about its mechanism of action. Nor is it possible to state whether the active principle is the same as that effective in extracts of the buffy-coat layer of horse blood.

SUMMARY

1. In guinea-pigs protected against a lethal histamine aerosol by an intraperitoneal injection of extract prepared from the buffy-coat layer of horse blood, the ileum, when suspended in a bath of tyrode solution, was found to be two to a thousand times less sensitive to histamine than a preparation obtained from an untreated animal. The sensitivity to acetylcholine was also reduced, but to a lesser degree.

2. An intraperitoneal injection of the extract was more effective than a subcutaneous one.

3. The reduced sensitivity of the ileum preparation was observed a few hours after the injection. When a purified extract prepared with Al_2O_3 column chromatograhy was used, the effect lasted for at least 24 hr.

4. Guinea-pigs were protected against a lethal histamine aerosol by intraperitoneal or subcutaneous injections of an alcoholic extract prepared from Hungarian oak gall.

5. In untreated guinea-pigs a 0·6–1 % histamine aerosol produces lethal bronchospasm, and the animals usually die within a few minutes. Protected animals withstood the histamine aerosol for 20 min or longer, and death when it occurred was apparently due to central respiratory failure.

6. These effects were obtained in unanaesthetized guinea-pigs and in guinea-pigs anaesthetized with urethane, in which the respiratory movements during the histamine aerosol were recorded on a smoked drum by transmission of the movements of the chest and abdomen to a lever through a hinged flap made of Perspex.

7. In unanaesthetized guinea-pigs protected by an injection of oak-gall extract the histamine aerosol produced not only changes in respiration but also in behaviour. The animals appeared to be partially paralysed or in a condition of light anaesthesia. This is probably a central effect of histamine, known to occur in other species, and unmasked in the guinea-pig because the lethal bronchospasm was prevented.

8. The protective effect of oak-gall extract developed within 1 hr after the injection; it lasted for 24 hr and sometimes as long as 4 days.

9. The protective effect of the oak-gall extract was not due to tannic acid. An injection of tannic acid was ineffective, but an extract from which the tannin had been removed by lead hydroxide precipitation produced the effect.

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