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HUMORAL FACTORS AFFECTING PULMONARY INFLATION DURING ACUTE ANAPHYLAXIS IN THE GUINEA-PIG *IN VIVO*

BY

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For more than 50 years, intense bronchoconstriction has been recognized as the cardinal feature of anaphylactic shock in the guinea-pig (Auer & Lewis, 1910; Biedl & Kraus, 1910). That the bronchoconstrictor response to antigen occurs without the nervous system (Auer & Lewis, 1910) and in isolated lung (Dale, 1920) supports the view that this response is mediated by substances released by the antigen-antibody reaction. The finding that histamine is liberated in anaphylaxis of isolated guinea-pig lung (Bartosch, Feldberg & Nagel, 1932), coupled with the knowledge of its powerful bronchoconstrictor action (Dale & Laidlaw, 1910) suggests that histamine is the mediator involved. This suggestion gains support from the findings that antihistamine drugs reduce the bronchoconstrictor response to antigen given either by injection (Staub & Bovet, 1937) or by inhalation (Armitage, Herxheimer & Rosa, 1952); but the incompleteness of this reduction suggests that either (1) released histamine is harder to antagonize than injected histamine, or (2) other mediators are also involved, as discussed in detail by Mongar & Schild (1962). The work described below was planned to test the hypothesis that other mediators as well as histamine are involved in anaphylactic bronchoconsriction in the guinea-pig, to identify as many of these as possible and to assess their role, using specific or selective antagonists.

One group of substances that seemed likely to play a part alongside histamine were the kinins, which are produced in anaphylaxis (Brocklehurst & Lahiri, 1962, 1963) and are powerful bronchoconstrictors (Collier, Holgate, Schachter & Shorley, 1959, 1960; Elliott, Horton & Lewis, 1960; Konzett & Stürmer, 1960; Bhoola, Collier, Schachter & Shorley, 1962) in the guinea-pig. A third humoral factor that might be involved was the slow-reacting substance (SRS-A) that is detected after anaphylaxis of guinea-pig isolated lungs (Kellaway & Trethewie, 1940; Brocklehurst, 1956, 1960; Chakravarty, 1960), a histamine-free preparation of which has bronchoconstrictor activity in this species (Berry & Collier, 1964).

The observation that aspirin and like-acting drugs antagonize bronchoconstriction induced both by kinins and SRS-A, without antagonizing that induced by histamine or several other endogenous substances (Collier & Shorley, 1960, 1963; Berry & Collier, 1964), enabled the role in the anaphylactic response of humoral factors antagonized in this way to be studied, although it did not allow particular factors to be identified.

Preliminary studies, using aspirin or the more potent antagonist, sodium meclofenamate, have been reported (Collier, Hammond & Whiteley 1963 ; Collier & James, 1966). That tachyphylaxis can be induced to bradykinin without inducing much cross-tachyphylaxis to SRS-A, and *vice versa* (Berry & Collier, 1964), enables the contribution of kinins and SRS-A to anaphylactic bronchoconstriction to be distinguished. The use of tachyphylaxis to estimate the contribution of kinins has been briefly reported (Collier & James, 1966). This report also indicated that adrenergic mechanisms play an important part in moderating the anaphylactic reaction. We describe more fully below these and further experiments to identify and to estimate the contribution of some humoral factors in anaphylactic bronchoconstriction of the guinea-pig.

METHODS

Materials

Table 1 gives the substances affecting tracheobronchial muscle that were studied, because they might be produced or released in anaphylaxis, and the main antagonists used to investigate their participation. Unless otherwise stated, doses of antagonists were those given in Table 1. The following salts were used: acetylcholine bromide, atropine sulphate, hexamethonium bromide, histamine acid phosphate, 5-hydroxytryptamine creatinine sulphate, mepyramine maleate, methysergide bimaleate, papaverine sulphate, tolazoline hydrochloride, pronethalol hydrochloride, propranolol hydrochloride, sodium meclofenamate, which is the salt of *N*-(2,6-dichloro-*m*-tolyl) anthranilic acid (Winder, Wax & Welford, 1965) and sodium acetylsalicylate. Weights of salts are expressed as active acid or base. Phenol (A.R.) was used. The bradykinin was synthesized by Nicolaides & DeWald (1961) and the decapeptide kallidin by Nicolaides, DeWald & McCarthy (1961). The SRS-A was " charcoal purified " material, prepared as previously described from the perfusate of isolated lungs of sensitized guinea-pigs (Berry & Collier, 1964), the lungs being perfused with antigen in Tyrode solution for 60–75 min. Samples of SRS-A were tested for freedom from histamine by intravenous injection into the Konzett-Rössler preparation of the guinea-pig, pretreated with pronethalol (10 mg/kg, subcutaneously) and aspirin (8 mg/kg, intravenously). For administration to sensitized guinea-pigs, SRS-A, dissolved in 0.9% NaCl in water, was boiled for 20 min to destroy the antigenicity of residual ovalbumen present (see Fig. 4). As antigen, ovalbumen (British Drug Houses) and zinc ovalbumen complex were used (see Fig. 2). Zinc ovalbumen complex was prepared by adding one part of $ZnCl_2$ to approximately four parts of ovalbumen in aqueous solution at pH 7.5. The precipitate was separated by centrifugation and repeatedly washed. Drugs were administered in solution or in suspension in 0.9% NaCl in water.

Sensitization

Six- to 8-week-old albino guinea-pigs (Duncan Hartley strain) were sensitized by injecting (1) 100 mg intraperitoneally and 100 mg subcutaneously of ovalbumen, and (2) 2 weeks later, a suspension of 100 mg zinc ovalbumen subcutaneously. During sensitization, animals were fed on a pellet diet (Oxoid modified 18) with supplementary greenstuffs and ascorbic acid. All animals used in experiments below were sensitized, unless described as " non-sensitized."

Experimental procedure

Five to 8 days after injecting zinc ovalbumen, animals were anaesthetized, deeply enough to suppress spontaneous breathing, with urethane (1.25–3.0 g/kg, intraperitoneally) or, when indicated in the text, with pentobarbitone sodium (60 mg/kg, intraperitoneally). They were prepared for recording air overflow volume by the method of Konzett & Rössler (1940), as modified by Collier *et al.* (1960). In this, the chest was unopened, the lungs were inflated with a miniature Starling pump at 72 strokes/min, at a constant stroke-volume between 5 and 9 ml., which was adjusted at the beginning of an experiment to give a minimal overflow volume. An increase in air overflow volume obtained in this way is taken to indicate bronchoconstriction. During recording, the side-vent of the recording

arm of the intratracheal cannula was clamped automatically for 10 sec during each 30 sec to inflate the lungs more forcibly (forced reinflation ; see Fig. 2), as described by Collier *et al.* (1963). During inflation, the pressure was 7–15 mm Hg and during reinflation 70–100 mm Hg. There was no added expiratory resistance.

Propranolol was given intraperitoneally (10 mg/kg) 30 min and intravenously (5 mg/kg) 5 min before challenge. Similar doses of pronethalol were used, except that in some experiments the intravenous dose was omitted. Other drugs were administered intravenously 5 min before challenge. After a reference dose of the agonist, the release of which was under investigation, animals were challenged with ovalbumen (0.25–10 mg/kg, intravenously), the dose being chosen to give a large submaximal response in the group of animals under test. Air overflow volume was recorded for 10 min after challenge. At the end of the experiment, the maximal increase of air overflow volume was recorded as the difference between the overall maximal value, obtained by clamping the trachea, and the base line value before challenge.

In some guinea-pigs, the brain and spinal cord were destroyed by pithing, after intratracheal cannulation under urethane anaesthesia. As an extra precaution in these animals, both vagi were cut in the neck. Adrenalectomy was performed after mid-line incision of the adbominal wall and ligation of the blood supply of the adrenal glands. As a control, sham adrenalectomy was performed.

As a guide to the amount of histamine or bradykinin that might participate in anaphylactic bronchoconstriction, mean dose/response lines were obtained for each substance in 5 animals pretreated with the same doses of antagonists as were used in control animals in the experiments in which the release of histamine or bradykinin was demonstrated.

Dose-ratios were determined by comparing the responses to an agonist, obtained 5 and 15 min after antagonist, with a dose-response line obtained before antagonist. Each value was the geometric mean of results in 5 guinea-pigs.

Design and interpretation of experiments

Experiments were designed so that animals to be used in the test group and their controls were taken randomly from the same batch of guinea-pigs, sensitized at the same time. In tests on each humoral factor, other possibly interfering humoral factors were antagonized by appropriate pretreatment. Treated and control animals were tested alternately. From the records of air overflow volume, the mean percent of maximum was plotted against time after challenge (see Fig. 7a). To derive the approximate contribution of the humoral factor antagonized, the differences of the mean time/response curves of the treated and control groups were plotted (see Fig. 7b).

To test the significance of drug effect at a given time, the percentage responses were subjected to an analysis of variance. In any one experiment, the common residual variance was used to test pairs of individual treatments (Davies, 1954). In some instances, to obtain minimal residual variance, the responses (x) were transformed before analysis to $\sqrt{x}$ or $\log_{10}(x+1)$ or $\sin^{-1}\sqrt{x}$. To test significance over a period of time, Σx was used.

RESULTS

Control and reference experiments

Recording. In 24 guinea-pigs pretreated with mepyramine, the effect of forced reinflation on the time/response curve after challenge with ovalbumen (10 mg/kg, intravenously) was investigated. Figure 1 shows that forced reinflation significantly ($P<0.01$) helped recovery.

Twenty guinea-pigs, pretreated with propranolol, tolazoline, methysergide, mepyramine and meclofenamate, were challenged with ovalbumen (4 mg/kg, intravenously). For 10 min after challenge, the pulmonary response was recorded with reinflation. From 5 min

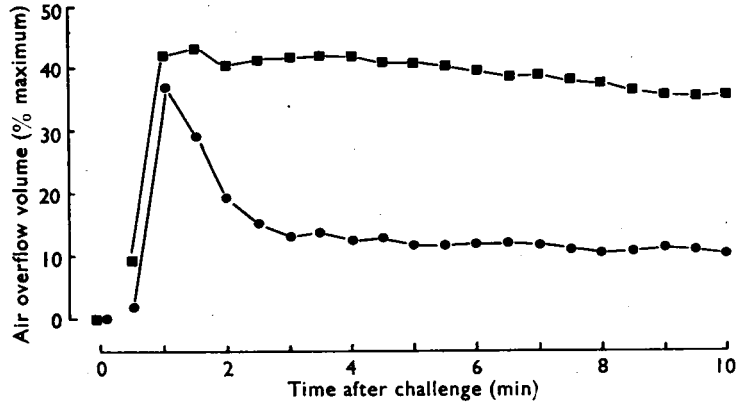

Fig. 1. Effect of forced reinflation in restoring the inflatibility of the lungs during anaphylaxis. Each curve is the mean of results in 12 guinea-pigs, anaesthetized with urethane (1.25–3.0 g/kg, intraperitoneally) and prepared for recording air overflow volume by the method of Konzett & Rössler (1940). Animals were sensitized with ovalbumen, 3 weeks, and with zinc ovalbumen, 1 week, before challenge. Mepyramine (2 mg/kg) was given intravenously 5 min before intravenous challenge with ovalbumen (10 mg/kg). ●——● with forced reinflation; ■——■ without forced reinflation. In forced reinflation, the side-vent of the intratracheal cannula is clamped automatically, to increase inflation pressure, for 10 sec during each 30 sec.

before to 10 min after challenge, the heart-beat was monitored with a Siemens Ediswan pen oscillograph. During this period, although the pulmonary response approached maximum the electrocardiogram remained normal. There was no sign of the heart-block that Auer & Lewis (1910) observed during the anaphylactic reaction of the guinea-pig, which they attributed to asphyxia.

Antigen. Intravenous administration of ovalbumen (1 or 10 mg/kg) had no effect on air overflow volume in Konzett-Rössler preparations of non-sensitized guinea-pigs; but, in animals sensitized by the procedure described above, ovalbumen at these doses induced a rapid increase of air overflow beginning 0.5–1 min after injection. A second dose of antigen was much less effective.

Figure 2 shows that a suspension of zinc ovalbumen, administered intravenously, was antigenic in guinea-pigs sensitized to ovalbumen. Because of this antigenicity, routine subcutaneous injection of a suspension of zinc ovalbumen (100 mg/kg) killed approximately 5% of guinea-pigs sensitized 14 days previously to ovalbumen. In four experiments involving 180 animals, sensitized with ovalbumen 3 weeks before challenge and pretreated with mepyramine, treatment with zinc ovalbumen 5–8 days before challenge intensified the early response to antigen ($P<0.001$). Figure 3 illustrates one of these experiments.

Of 24 guinea-pigs sensitized with ovalbumen, 12 received zinc ovalbumen complex two weeks later. Five days afterwards, all animals were challenged intravenously with 0.5 mg/kg ovalbumen. The mean response over 10 min of animals that had received zinc ovalbumen was very significantly ($P<0.001$) less than that of animals that did not receive zinc ovalbumen.

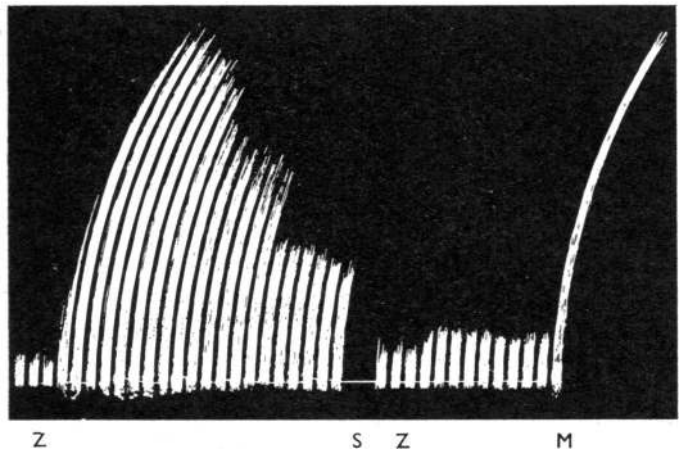

Fig. 2. Antigenicity of zinc ovalbumen. Guinea-pig, 520 g, sensitized and prepared, with forced reinflation, as in Fig. 1. Z, zinc ovalbumen complex (10 mg/kg I.V.); S, drum stopped for 10 min; M, maximal air overflow volume with trachea clamped (8.5 ml.).

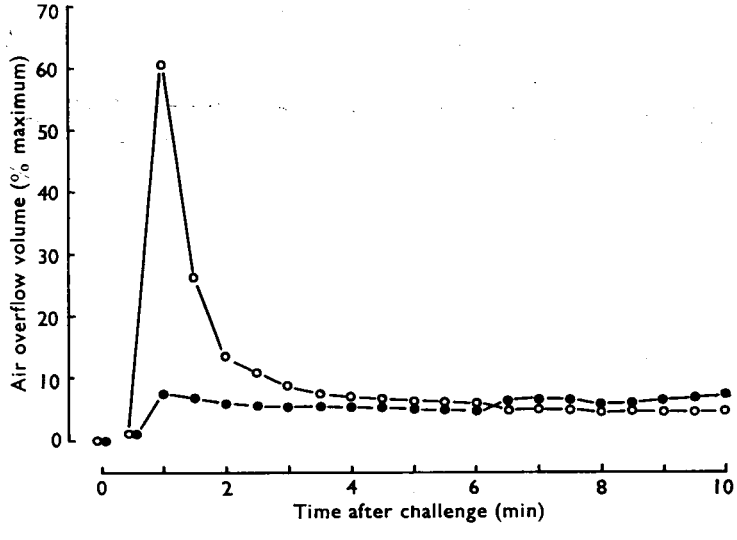

Fig. 3. Effect of zinc ovalbumen on sensitization. Each curve is the mean of results in 8 guinea-pigs, prepared with forced reinflation, as in Fig. 1. Animals were sensitized with ovalbumen, 3 weeks, and treated with mepyramine (2 mg/kg I.V.), 5 min, before intravenous challenge with ovalbumen (10 mg/kg). ○——○ zinc ovalbumen complex (100 mg, subcutaneously), 6 days before challenge; ●——● not treated with zinc ovalbumen.

Anaesthetic. Since high concentrations of urethane lessen the amount of histamine released from guinea-pig lung by the antigen-antibody reaction *in vitro* (Mongar & Schild, 1957), we tested whether the anaphylactic response *in vivo* was greater when pentobarbitone replaced urethane as anaesthetic. Of 24 guinea-pigs pretreated with propranolol, 12 animals were anaesthetized with pentobarbitone and 12 with urethane (3 g/kg intraperitoneally). The mean time/response curves of the two groups were of the same shape and, although that of urethane was slightly lower than that of pentobarbitone, the curves did not differ significantly in height.

SRS-A. Since charcoal purified SRS-A contained enough antigen to elicit anaphylaxis, solutions of SRS-A were boiled for 20 min before administration to sensitized guinea-pigs. Figure 4 shows that samples retained bronchoconstrictor activity after this procedure. Both boiled and unboiled SRS-A were antagonized by acetylsalicylate or meclofenamate and showed cross-tachyphylaxis. Boiling for 20 min destroyed the antigenicity of egg albumen as expected from the early literature (Dale, 1920).

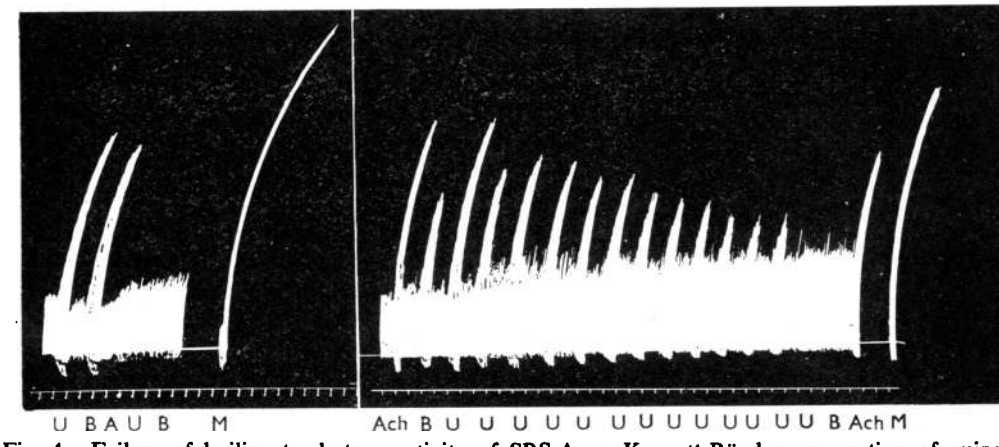

Fig. 4. Failure of boiling to destroy activity of SRS-A on Konzett-Rössler preparation of guinea-pig lungs *in vivo*. Time, 30 sec; M, maximal air overflow volume. Both animals were non-sensitized and were pretreated with pronethalol (10 mg/kg, intraperitoneally+5 mg/kg I.V.). Left-hand tracing, guinea-pig, 460 g. U, unboiled SRS-A (0.2 mg); B, SRS-A (0.2 mg) boiled for 20 min; A, sodium acetylsalicylate (8 mg/kg); M, 8 ml. Right-hand tracing, guinea-pig, 670 g. Ach, acetylcholine (5 μg); B, SRS-A (0.3 mg) boiled for 20 min; U, unboiled SRS-A (0.3 mg); M, 6.5 ml. Test drugs were given intravenously.

Antagonists. Table 1 gives the doses of antagonists used and the dose-ratios, at the times corresponding with challenge (5 min) and with the end of recording (15 min), of atropine, methysergide, mepyramine and meclofenamate, when each drug acted against the same background of treatment as in the test on anaphylactic bronchoconstriction described below. Table 1 also gives the dose of bradykinin or SRS-A used to induce tachyphylaxis. Between six and 15 doses, given as frequently as practicable, were needed. Where tachyphylaxis to bradykinin or SRS-A was used, the conditions in anaphylactic experiments could not be duplicated in reference experiments of the type summarized in Table 1. In each of two experiments, tachyphylaxis to bradykinin was accompanied by cross-tachyphylaxis to kallidin.

TABLE 1

DOSES OF SPECIFIC OR SELECTIVE ANTAGONISTS USED AND THE POTENCIES OF SOME OF THESE IN THE CONDITIONS OF THE EXPERIMENTS

The dose-ratio is the ratio of equiactive doses of agonist administered after and before the stated dose of antagonist. Dose-ratios were assessed in the Konzett–Rössler preparation of guinea-pig lungs *in vivo*, at 5 and 15 min after intravenous injection of antagonist. Each value is the geometric mean of 5 results. The dose ratio of an antagonist was obtained with the background of treatment used in tests to detect the corresponding agonist in anaphylactic bronchoconstriction. Propranolol and pronethalol were administered intraperitoneally (I.P.) 30 min and intravenously 5 min before agonist. Tachyphylaxis to kinins was induced by repeated injection of bradykinin (20 μg I.V.) and to SRS-A by repeated injection of SRS-A (1 mg I.V.). Meclofenamate is the sodium salt of *N*-(2,6-dichloro-*m*-tolyl)anthranilic acid. –, not tested; rep., repeated injection. Except where tachyphylaxis was induced to bradykinin or SRS-A, doses are in mg/kg.

Humoral factor	Antagonist	Dose	Dose ratio at 5 min	Dose ratio at 15 min
Catechol amines	Tolazoline	5 I.V.	—	—
	Propranolol	10 I.P.+5 I.V.	—	—
	Pronethalol	10 I.P.+5 I.V.	—	—
Acetylcholine	Atropine	1 I.V.	288	179
	Hexamethonium	5 I.V.	—	—
5-Hydroxytryptamine	Methysergide	0·1 I.V.	48	35
Histamine	Mepyramine	2 I.V.	642	477
Kinins	Bradykinin	20 μg I.V. rep.	—	—
	Meclofenamate	1 I.V.	92	87
SRS-A	SRS-A	1 mg I.V. rep.	—	—
	Meclofenamate	1 I.V.	20	13

To test whether the antagonists in Table 1 altered the base-line of response, they were injected into each of 5 animals, at the doses and by the routes shown in that Table. In no animal was the base-line of response significantly altered by this treatment.

Destruction of the central nervous system. In 12 of 24 guinea-pigs, the brain and spinal cord were destroyed 5 min before intravenous challenge, the remaining 12 animals serving as controls. In accordance with the finding of Auer & Lewis (1910), no significant difference in mean air overflow between the two groups was obtained at any point in the time/response curves.

In 20 of 40 guinea-pigs, the brain and spinal cord were destroyed 10 min before intravenous challenge and in the remaining 20 animals the central nervous system was left intact. Five minutes later, groups of 5 animals received intravenously (1) 0.9% NaCl in water (2) mepyramine, (3) meclofenamate (0.4 mg/kg), or (4) mepyramine and meclofenamate (0.4 mg/kg). Figure 5 shows that animals with or without a central nervous system responded to challenge with antigen in a comparable way for the different test drugs, although after pithing and meclofenamate the responses to challenge seemed slower.

Acetylsalicylate and phenol. In two experiments involving 63 guinea-pigs, similar in design to that illustrated in Fig. 5, except that the brain and spinal cord were left intact, acetylsalicylate (16 mg/kg, intravenously) was used instead of meclofenamate. Acetylsalicylate suppressed the same part of the response to antigen as did meclofenamate. In guinea-pigs pretreated with propranolol, phenol (8 mg/kg intravenously) did not reduce significantly the mean response to antigen of 12 animals, compared with that of 12 controls.

Bronchodilator factors

Bilateral adrenalectomy 15 min before challenge, or β-receptor blockade, greatly intensified and prolonged the response of sensitized guinea-pigs to intravenous antigen

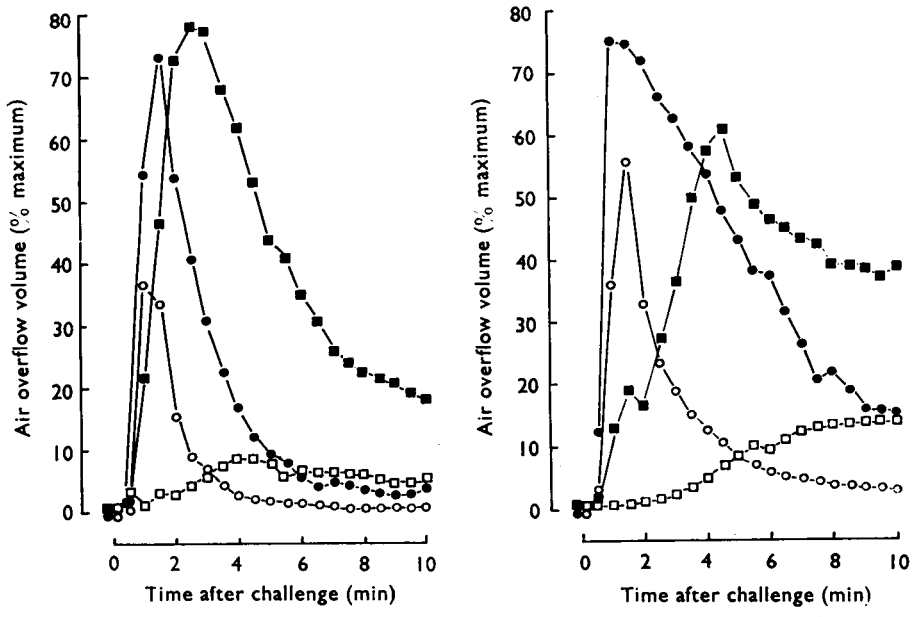

Fig. 5. Effect of mepyramine and meclofenamate on anaphylactic bronchoconstriction in guinea-pigs with C.N.S. intact or destroyed. Each curve is the mean of results in 5 animals, sensitized and prepared, with forced reinflation, as in Fig. 1. In the left-hand figure, the brain and spinal cord were intact; in the right-hand figure, the brain and spinal cord were destroyed by pithing 10 min before challenge. ●——●, 0.9% NaCl in water; ○——○, mepyramine (2 mg/kg); ■——■, meclofenamate (0.4 mg/kg); □——□, mepyramine (2 mg/kg)+meclofenamate (0.4 mg/kg). Test drugs were given intravenously 5 min before intravenous challenge with ovalbumen. Ovalbumen (10 mg/kg) was used in the group receiving both mepyramine and meclofenamate; ovalbumen (2 mg/kg) was used in the other 3 groups.

(Fig. 6). In this figure, based on an experiment in 72 guinea-pigs, the difference of mean time/response curves of animals subjected or not subjected to adrenalectomy is significant ($P<0.001$). The effect of propranolol is significant compared with no treatment ($P<0.001$) or with adrenalectomy ($P<0.005$). The effect of pronethalol is significant compared with no treatment ($P<0.001$), but not compared with adrenalectomy. Tolazoline (5 mg/kg intravenously) appeared to enhance the effects of propranolol or pronethalol, but the difference was not significant. In the experiments below to identify humoral bronchoconstrictor factors, adrenergic blockade was used to reduce uncontrolled sympathomimetic effects.

Bronchoconstrictor factors

Cholinergic. In an experiment in 24 guinea-pigs pretreated with propranolol, and in another experiment in 24 guinea-pigs, pretreated with propranolol and tolazoline, atropine did not reduce the anaphylactic response. In an experiment in 24 guinea-pigs, pretreated with propranolol and tolazoline, hexamethonium also failed to reduce the anaphylactic response.

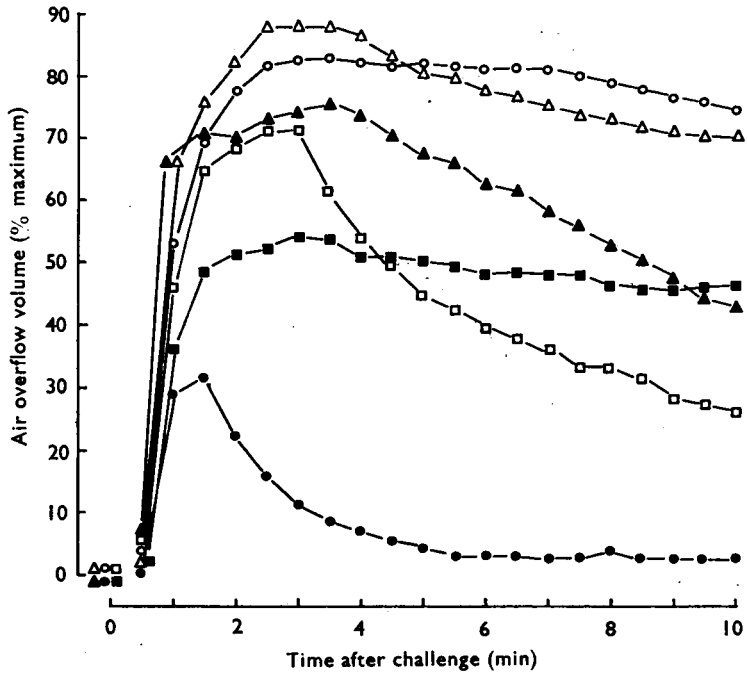

Fig. 6. Intensification of anaphylactic bronchoconstriction by adrenalectomy or adrenergic blockade. Each curve is the mean of results in 12 guinea-pigs, sensitized and prepared, with forced reinflation, as in Fig. 1. ●——● 0.9% NaCl in water; ○——○ propranolol; □——□ pronethalol; Δ—— Δ propranolol+tolazoline; ▲——▲ pronethalal+tolazoline; ■——■ adrenalectomy. Propranolol or pronethalol was given 30 min (10 mg/kg, intraperitoneally) and 5 min (5 mg/kg I.V.) before intravenous challenge with ovalbumen (4 mg/kg). Tolazoline (5 mg/kg I.V.) was given 5 min before challenge. Bilateral adrenalectomy was performed 15 min before challenge.

5-Hydroxytryptamine. In guinea-pigs, pretreated with propranolol or pronethalol, and with tolazoline, mepyramine and meclofenamate, we tested how far methysergide lessened the response to intravenous antigen. In only two of seven experiments, involving a total of 168 animals, was methysergide significantly ($P<0.05$) effective. Although this suggested that 5-hydroxytryptamine usually played little part in mediating anaphylactic bronchoconstriction, methysergide was used in experiments on other bronchoconstrictor factors, in case release of 5-hydroxytryptamine should occur.

Histamine. In guinea-pigs, pretreated with propranolol or pronethalol and with tolazoline, methysergide and meclofenamate, we tested how far mepyramine lessened the response to intravenous antigen. In all of three experiments, involving 72 animals, mepyramine reduced the intensity of the response. Figure 7a shows the mean time/response curves obtained with and without mepyramine in one experiment with 24 animals. The difference between these curves, plotted in Fig. 7b, was significant ($P<0.005$). For reasons discussed below, this difference is taken to represent approximately a contribution of histamine to the anaphylactic response. At its peak, 2.5 min after challenge, this contribution corresponded in intensity with 0.54 μg/kg histamine, injected intravenously after adrenergic blockade.

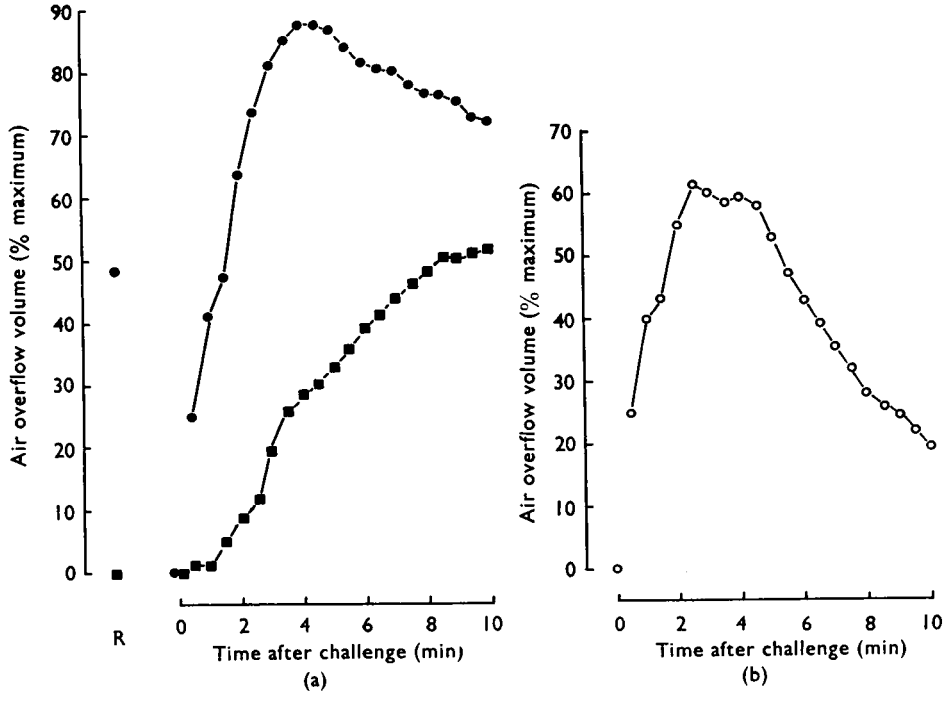

Fig. 7. Reduction of anaphylactic bronchocontriction by mepyramine. Each curve is the mean of results in 12 guinea-pigs, sensitized and prepared, with forced reinflation, as in Fig. 1. All animals were pretreated with pronethalol and tolazoline as in Fig. 6. They also received methysergide (0.1 mg/kg) and meclofenamate (1 mg/kg) intravenously 5 min before intravenous challenge with ovalbumen (4 mg/kg). Fig. 7a: R, reference dose of histamine (0.5 μg I.V.) I min before challenge; ●——●, 0.9% NaCl in water; ■——■, mepyramine (2 mg/kg). NaCl or mepyramine was given intravenously 5 min before challenge. Fig. 7b: the difference between the curves in Fig. 7a.

Kinins and SRS-A. In guinea-pigs pretreated with pronethalol, methysergide and mepyramine, we tested how far meclofenamate lessened the response to intravenous antigen. In all of four experiments, involving 96 animals, meclofenamate reduced the intensity of the response.

In 36 guinea-pigs, pretreated with pronethalol, methysergide and mepyramine, the effect of tachyphylaxis to bradykinin was compared with the effects of meclofenamate and of no treatment. Figure 8a gives the resulting mean time/response curves. At its peak, 1.5 min after challenge, the control curve is significantly higher than the curve after bradykinin tachyphylaxis ($P<0.005$) and than that after meclofenamate ($P<0.001$). At its peak, the curve after tachyphylaxis is significantly higher than that after meclofenamate ($P<0.025$). Figure 8b gives the differences between the time/response curves in Fig. 8a, indicating (curve K) the part of the acute anaphylactic response removed by tachyphylaxis to bradykinin; (curve K+S) the part removed by meclofenamate; and (curve S) the difference between them, representing those factors, other than kinins, that are antagonized by meclofenamate. For reasons discussed below, the time/difference curve K in

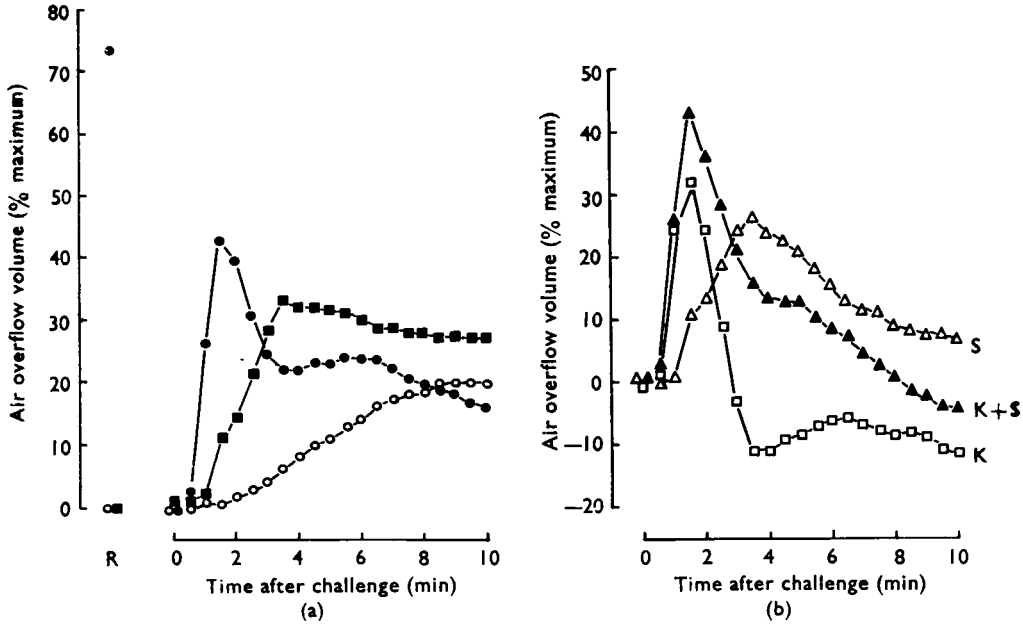

Fig. 8. Reduction of anaphylactic bronchoconstriction by tachyphylaxis to bradykinin and by meclofenamate. Each curve is the mean of results in 12 guinea-pigs sensitized and prepared, with forced reinflation, as in Fig. 1. All animals were pretreated with pronethalol (10 mg/kg, intraperitoneally) 30 min before challenge with ovalbumen (4 mg/kg I.V.). They also received methysergide (0.1 mg/kg) and mepyramine (2 mg/kg) intravenously 5 min before challenge. Fig. 8a: R, reference dose of bradykinin (0.1 μg I.V.) I min before challenge; ●——●, 0.9% NaCl in water; ○——○ meclofenamate (1 mg/kg); ■——■, bradykinin (20 μg I.V.) repeated until the preparation was unresponsive. NaCl or meclofenamate was given intravenously 5 min before challenge. Fig. 8b: the differences between the curves in Fig. 8a. □——□, between braykinin tachyphylaxis and NaCl; ▲——▲, between meclofenamate and NaCl; Δ——Δ between bradykinin tachyphylaxis and meclofenamate. The curve K represents a contribution of kinins, the curve S of SRS-A and the curve K+S of both to anaphylactic bronchoconstriction (see Discussion).

Fig. 8b is taken to indicate approximately a contribution of kinins to the anaphylactic response. At its peak, this contribution corresponded in intensity with the effect of about 0.09 μg/kg bradykinin, injected intravenously after adrenergic blockade.

In another 36 guinea-pigs, also pretreated with pronethalol, methysergide and mepyramine, the effect of tachyphylaxis to SRS-A was compared with the effects of meclofenamate and of no treatment. Two of 12 animals that failed to become completely unresponsive to 1 mg/kg SRS-A were omitted from the analysis. Figure 9a gives the resulting mean time/response curves. At its peak, the control curve is significantly higher than the curve after SRS-A tachyphylaxis ($P=0.05$) and than that after meclofenamate ($P<0.005$). In its early stages, the curve after tachyphylaxis is significantly higher than that after meclofenamate ($P<0.025$). Figure 9b gives the differences between the time/response curves in Fig. 9a, indicating (curve S) the part of the acute anaphylactic response removed by tachyphylaxis to SRS-A, approximately representing a contribution of SRS-A; (curve K+S) the part removed by meclofenamate; and (curve K) the

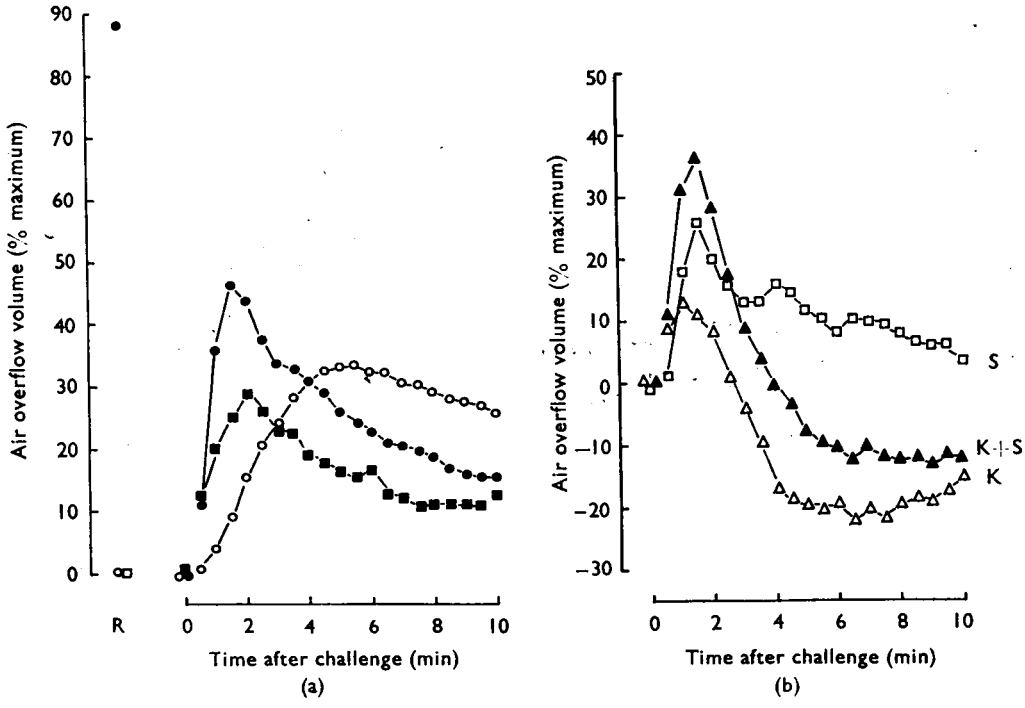

Fig. 9. Reduction of anaphylactic bronchoconstriction by tachyphylaxis to SRS-A and by meclofenamate. All preparations, treatments and symbols are as in Fig. 8a and b, except that SRS-A (1 mg) replaces bradykinin and that only 10 guinea-pigs are represented in the curve for tachyphylaxis to SRS-A. The curves K, S and K+S are lettered as in Fig. 8b.

difference between these parts, representing those factors, other than SRS-A, that are antagonized by meclofenamate. In both Fig. 8b and Fig. 9b, the peak of curve K occurs earlier than that of curve S.

Residual bronchoconstriction. Figure 7a shows that, after α- and β-receptor blockade, in spite of treatment with methysergide, mepyramine and meclofenamate, a residual bronchoconstriction developed, beginning 1.5 min after injection of antigen. Figure 5 shows that the residual bronchoconstriction was slight, however, when mepyramine and meclofenamate were not accompanied by adrenergic blockade. A further experiment in 36 guinea-pigs, pretreated with mepyramine and meclofenamate, showed that pronethanol was about equally effective in intensifying ($P<0.01$) the residual bronchoconstriction in animals with the central nervous system intact or destroyed (Fig. 10). In an experiment on 24 guinea-pigs pretreated with propranolol, methysergide, mepyramine and meclofenamate, papaverine (5 mg/kg intravenously) significantly ($P<0.025$) reduced the residual response.

In some animals, for 10 min after challenge, the pressure developed in the trachea during forced reinflation was measured with a Statham transducer connected to a Devices polygraph. The highest reinflation pressure recorded was 102 mm Hg. To test whether the residual bronchoconstriction might be due to mechanical damage to the lungs resulting from high reinflation pressure, experiments were performed in 84 sensitized animals,

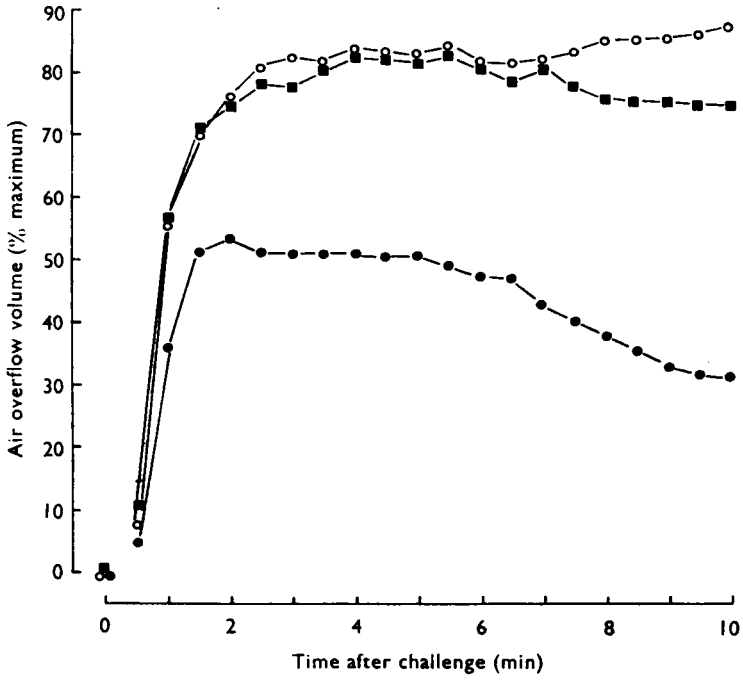

Fig. 10. Intensification of residual bronchoconstriction by pronethalol in guinea-pigs in which the C.N.S. had been destroyed. Each curve is the mean of results in 12 animals sensitized and prepared, with forced reinflation, as in Fig. 1. All animals were pretreated with mepyramine (2 mg/kg) and meclofenamate (1 mg/kg) intravenously 5 min before intravenous challenge with ovalbumen (10 mg/kg). ●——● 0.9% NaCl in water; ■——■ pronethalol (10 mg/kg, intraperitoneally) 30 min before challenge; ○——○ pronethalol (10 mg/kg, intraperitoneally) 30 min, and C.N.S. destroyed, 15 min, before challenge.

pretreated with tolazoline, propranolol, methysergide, mepyramine and meclofenamate, in which the lungs were reinflated at pressures of 10, 15, 20 or 25 mm Hg, controlled by a water valve in the recording unit. No significant reduction was observed in the intensity of the residual bronchoconstriction, compared with that in control animals reinflated at the usual pressures.

DISCUSSION

The ability of SRS-A, prepared as described by Berry & Collier (1964), to retain activity after boiling for 20 min (Fig. 4) was unexpected. We do not know whether the SRS-A of Brocklehurst (1956, 1960) also possesses this property, since preliminary experiments carried out jointly with Brocklehurst and Marquis suggested that the material used in the experiments described above differs from Brocklehurst's material in having greater activity on guinea-pig bronchial muscle and less on guinea-pig ileum. The lower dose-ratio of meclofenamate against SRS-A than against bradykinin (Table 1) may be an intrinsic difference indicating a more intense antagonism of bradykinin or, alternatively, it may be due to some impurity in SRS-A, not antagonized by meclofenamate and exerting bronchoconstrictor action at higher doses.

The failure, in our experiments, of atropine to reduce anaphylactic bronchoconstriction in guinea-pigs pretreated with adrenergic blocking agents did not accord with the statements of Auer & Lewis (1910) and of Alberty (1959) that atropine reduced the bronchoconstrictor response to intravenous antigen, nor with the finding of Armitage *et al.* (1952) that atropine exerted a protective action against inhaled antigen aerosol. Our failure, however, was consistent with that of Brocklehurst (1958), who could not detect acetylcholine in the perfusate of the isolated lungs of sensitized guinea-pigs, challenged with antigen, and with the report of Auer (1910) that cutting and degeneration of the vagi did not lessen the intensity of anaphylactic bronchoconstriction, although Auer confirmed the finding that a large dose of atropine (3 mg) reduced the response. The difference between our experience with atropine and that of the above authors may perhaps be due to the difference in our sensitization procedure, in the use of zinc ovalbumen complex a week before challenge, or to our pretreatment with a β-receptor antagonist, or again to our use of animals that were deeply anaesthetized.

Our failure to obtain consistent evidence of the participation of 5-hydroxytryptamine in anaphylactic bronchoconstriction accords with the general conclusion in the guinea-pig (Herxheimer, 1955a ; Brocklehurst, 1958 ; Udenfriend & Waalkes, 1959 ; Mongar & Schild, 1962).

The finding that adrenalectomy intensified and prolonged the bronchconstrictor response to antigen accords with previous observations in the guinea-pig (Kepinow, 1922 ; Gross & Haefeli, 1952) and in other species (Rose, 1959). Our experiments do not determine whether this effect is due to catechol amines or to corticosteroids ; but that pronethalol or propranolol has as great an effect as adrenalectomy or greater (Fig. 6) indicates that catechol amine release is probably the main factor in the conditions of the present experiments, as might be expected where adrenalectomy was performed only 15 min before challenge. That intravenous injection of antigen liberates catechol amines into the circulation of the sensitized guinea-pig for 15 to >60 min has now been demonstrated with the blood-bathed organ technique (Piper, Collier & Vane, 1967).

The greater effect of propranolol than adrenalectomy (Fig. 6) also suggests that another adrenergic factor is involved. This may be the stimulation of sympathetic ganglia (Lewis & Reit, 1965 ; Trendelenburg, 1966). At least a part of the release of catechol amines from the adrenal glands and of the possible stimulation of sympathetic nerves may well be due to a direct action on these structures by bradykinin and other substances released by the antigen-antibody reaction. The moderating effect of adrenergic factors on anaphylactic bronchoconstriction was so great that, to avoid deaths in antagonism experiments, the milder blocking agent pronethalol was usually preferred to propranolol. The intrinsic sympathomimetic activity of pronethalol (Black, Duncan & Shanks, 1965) may also have helped to reduce its lethality.

The experiments illustrated in Figs. 5 and 7 show that mepyramine suppresses a part of anaphylactic bronchoconstriction. Mepyramine does not antagonize bronchoconstriction induced in the guinea-pig by bradykinin (Collier & Shorley, 1960), SRS-A (Berry & Collier, 1964), acetylcholine or 5-hydroxytryptamine (Holgate & Warner, 1960) ; but it readily antagonizes that induced by injected histamine (Bovet, Horclois & Walthert, 1944 ; Holgate & Warner, 1960). Histamine is known to be released by the antigen-antibody reaction in guinea-pig lung (Bartosch *et al.*, 1932). Our finding with mepyramine,

therefore, confirms the accepted view that released histamine participates in anaphylactic bronchoconstriction in the guinea-pig.

The experiments illustrated in Figs. 5, 8 and 9 show that there is another part of anaphylactic bronchoconstriction, which is suppressed by meclofenamate. Meclofenamate, like acetylsalicylate and other antipyretic drugs, antagonizes bronchoconstriction induced by bradykinin, SRS-A and ATP, but not that induced by acetylcholine, histamine or 5-hydroxytryptamine (Collier & James, 1966 ; Collier, James & Schneider, 1966). Kinins (Brocklehurst & Lahiri, 1962, 1963 ; Jonasson & Becker, 1965 ; Greeff, Scharnagel, Lühr & Strobach, 1966) and SRS-A (Brocklehurst, 1956, 1960 ; Chakravarty, 1960 ; Berry & Collier, 1964) are released in anaphylaxis of the guinea-pig. The possibility that ATP is released in anaphylaxis depends mainly on experiments in the dog (Shibusawa, Tokuzawa, Kishi, Kajiya, Kasuga & Fujiwara, 1958a and b ; Back, Wilkens & Steger, 1966). The simplest explanation, therefore, of the effect of meclofenamate is that it reduces anaphylactic bronchoconstriction by antagonizing kinins, SRS-A and possibly ATP ; but the question of the relative contributions of these three substances remains to be considered.

Before discussing the relative contributions of kinins, SRS-A and ATP to anaphylactic bronchoconstriction, the possibility must be considered that meclofenamate acts by suppressing some earlier phase of the antigen-antibody reaction, rather than by antagonizing a released humoral factor. The evidence against this is based partly on analogy with acetylsalicylate. According to Mongar & Schild (1957), about 10 mg/ml. acetylsalicylate or salicylate is needed to reduce by 50% the release of histamine by antigen from sensitized lung *in vitro*. According to Trethewie (1951), 0.25 mg/ml. acetylsalicylate is required to suppress the release of histamine from guinea-pig isolated lung perfused with antigen. Such concentrations are much higher than that of 56 μg/ml. total salicylate obtained 0.5 min after intravenous injection of 4 mg/kg acetylsalicylate (Whitely & James, unpublished), a dose that is effective in reducing anaphylactic bronchoconstriction (Collier, *et al.*, 1963). This dose is, in turn, twice the minimal effective dose against bronchocontriction induced by injected bradykinin or SRS-A (Collier & Shorley, 1960, 1963 ; Berry & Collier 1964). Whereas acetylsalicylate readily suppresses a part of anaphylactic bronchoconstriction, phenol, which Mongar & Schild (1957) found to be 15 times as potent as acetylsalicylate in inhibiting histamine release from shocked guinea-pig lung *in vitro*, failed to reduce any part of anaphylactic bronchoconstriction *in vivo* at a dose equal to an effective dose of acetylsalicylate.

Meclofenamate resembles acetylsalicylate in pharmacological properties, but is more potent (Winder *et al.*, 1965 ; Collier *et al.*, 1966). Meclofenamate suppresses a part of anaphylactic bronchoconstriction at 0.4–1 mg/kg, intravenously, as reported above and by Collier & James (1966). This dose range is 3–8 times the minimal effective dose against bronchoconstricton induced by injected bradykinin or SRS-A. By analogy with acetylsalicylate, therefore, meclofenamate may be supposed to act in anaphylaxis by antagonism of released kinins, SRS-A and possibly ATP. This conclusion accords with the finding that meclofenamate suppressed a different part of the anaphylactic response from that suppressed by mepyramine (Fig. 5).

Two control experiments reported above confirm that cross-tachyphylaxis develops between bradykinin and kallidin. Berry & Collier (1964) and Collier *et al.* (1966) have

reported experiments in which tachyphylaxis was induced to bradykinin, SRS-A or ATP and the accompanying cross-tachyphylaxis tested. These experiments showed that (1) animals in which tachyphylaxis was induced to bradykinin remained responsive to SRS-A and ATP; (2) animals in which tachyphylaxis was induced to SRS-A remained responsive to bradykinin and ATP; and (3) animals in which tachyphylaxis was induced to ATP remained responsive to bradykinin, but were unresponsive to SRS-A. In the light of these findings, the experiment illustrated in Fig. 8 may be interpreted to show that kinins participate in anaphylactic bronchoconstriction in the guinea-pig, their contribution being approximately represented by the curve K in Fig. 8b, which is the difference between the time/response curves after saline and after bradykinin tachyphylaxis. The experiment in Fig. 9b likewise indicates that SRS-A participates, its contribution being approximately represented by the curve S.

The sum of the contributions of bradykinin (Fig. 8b) and of SRS-A (Fig. 9b) corresponds roughly with the mean of the two curves for meclofenamate (Fig. 8b and 9b). This leaves little room for ATP as a factor in anaphylactic bronchoconstriction in the guinea-pig. This is not surprising, in view of its very low bronchoconstrictor potency when injected intravenously (Collier *et al.*, 1966) and of the failure of Kitamura (1965) to demonstrate release of ATP from lung during anaphylaxis in the guinea-pig.

Since there is little room in the action of meclofenamate for the antagonism of factors other than the kinins and SRS-A, the curve S in Fig. 8b, being the difference between the time/response curves after meclofenamate and after tachyphylaxis to bradykinin, is taken to represent approximately a contribution of SRS-A. Likewise, the curve K in Fig. 9b is taken to represent approximately a contribution of bradykinin. If these inferences are correct, the bradykinin-induced bronchoconstriction would appear to reach its peak slightly sooner than that of SRS-A.

In the experiments of Smith & Humphrey (1949) and of Herxheimer (1955b), sodium salicylate failed to reduce anaphylactic bronchoconstriction in conscious guinea-pigs. An attempt to show this effect, using acetylsalicylate in a few anaesthetized guinea-pigs also failed (Collier & Shorley, 1960). Using a large number of animals, however, Collier *et al.* (1963) showed that intravenous aspirin significantly reduced anaphylactic bronchoconstriction in anaesthetized guinea-pigs. In their experiments, an intravenous dose of 1 μg histamine was given 5 min before challenge, to test the responsiveness of the bronchial muscle. The present results, obtained with aspirin or meclofenamate, in animals treated with zinc ovalbumen 5–8 days before challenge, confirm and extend the finding. Injection of zinc ovalbumen produced animals in which the effect of aspirin or meclofenamate was more evident (Fig. 3), perhaps because the ratio of kinins to histamine may have been increased. In the earlier experiments of Collier *et al.* (1963), the intravenous injection of histamine before challenge may have had a comparable effect, as Edery & Lewis (1963) showed in the dog, in tissue injury.

That mepyramine and meclofenamate together antagonize most of the increased air overflow in anaphylaxis, in animals with functioning adrenergic mechanisms (Fig 5), confirms the previous report of Collier *et al.* (1963) on mepyramine and acetylsalicylate. After adrenergic blockade, all phases of the anaphylactic response are intensified, including the residue not antagonized by mepyramine and meclofenamate (Figs 7, 8, 9 and 10). That papaverine lessens this residue suggests that bronchospasm is at least partly

responsible. The cause of the residual bronchoconstriction is obscure; but, from the experiments reported, it does not appear to depend on high reinflation pressure nor on the integrity of the brain and spinal cord. The residual effect might be due to a humoral factor, either a substance already implicated but not completely antagonized, through inaccessibility or other reason, or to a different substance altogether. The residual response might reflect cellular damage; it might be related to fluid changes as well as to smooth muscle contraction in the lung. We believe that the residual bronchoconstriction presents an important problem for study.

Bronchial asthma in man shares some common features with anaphylactic bronchoconstriction in the guinea-pig. For example, propranolol intensifies asthma (McNeill, 1964; Meier, Lydtin & Zöllner, 1966) as it does the guinea-pig reaction. Again, SRS-A probably plays a part in asthma (Brocklehurst, 1956; Herxheimer & Stresemann, 1963) as in guinea-pig anaphylaxis. How far other findings in the guinea-pig, such as the anti-anaphylactic action of meclofenamate and the existence of a bronchoconstriction not antagonized by mepyramine or meclofenamate, apply in man remains to be explored.

SUMMARY

1. The participation of humoral factors in acute anaphylactic bronchoconstriction has been investigated, by means of specific or selective antagonists, in the Konzett–Rössler preparation of guinea-pig lungs *in vivo*.

2. Aspirin, or the more potent like-acting drug meclofenamate, suppressed a part of the immediate response to intravenous antigen; mepyramine suppressed another part. Together, meclofenamate and mepyramine suppressed most, but not all, of the response, whether the central nervous system was intact or destroyed.

3. Bilateral adrenalectomy, or, still more, adrenergic blockade intensified and prolonged the response. Adrenergic blockade was effective also in animals in which the central nervous system had been destroyed.

4. After adrenergic blockade, atropine or hexamethonium did not significantly reduce anaphylactic bronchoconstriction and the effect of methysergide was doubtful, whereas meclofenamate and mepyramine remained effective.

5. Since meclofenamate antagonizes kinins, slow-reacting substance in anaphylaxis (SRS-A) and ATP, the contributions of these factors were determined by inducing tachyphylaxis separately to bradykinin and to SRS-A. This showed that both kinins and SRS-A contribute to anaphylactic bronchoconstriction, and that the sum of their effects accounts for the total effect of meclofenamate.

6. We conclude that kinins, SRS-A and histamine participate in acute anaphylactic bronchoconstriction in the guinea-pig. Release of catechol amines during the reaction moderates the bronchoconstriction. Another unknown bronchoconstrictor factor(s) is also involved.

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