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THE ANTAGONISM OF HISTAMINE AND THE ANAPHYLACTIC RESPONSE BY PHENYLPYRIDYLALLYLAMINES

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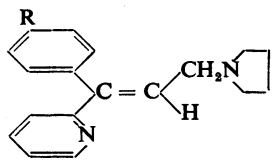
A moderate degree of antihistamine activity was found in a series of diphenylallylamines (White, Green, and Hudson, 1951) and in some dithienylallylamines (Green, 1953), and investigation of the related phenylpyridylallylamines made by Adamson and Billinghurst (1950) has shown that many of these latter compounds are powerful antihistamines. They can exist in two steroisomeric forms. The antihistamine activity is confined mainly to the *trans* isomer, as in *trans*-l-(4′-chlorophenyl)-l-(2′-pyridyl)-3-pyrrolidinoprop-l-ene maleate (Adamson, Barrett, Billinghurst, Green, and Jones, 1951). This compound, known by the code number 405C49, has been shown to have a powerful antihistamine action in man (Bain, 1951).

A description of the pharmacological properties of 405C49 and the most active member of this series, *trans*-l-(4′-methylphenyl)-l-(2′-pyridyl)-3-pyrrolidinoprop-l-ene hydrochloride (295C51) (Adamson, Barrett, Billinghurst, and Jones, 1953), in laboratory animals is presented in this paper.



In 405C49, R=Cl; in 295C51, R=CH_3

RESULTS

Effects on Histamine Action

Guinea-pig Ileum.—The compounds were compared for relaxation of histamine-induced spasm of isolated guinea-pig ileum by the method described previously (Green, 1953). The activities, relative to that of 405C49, were calculated from the percentage relaxation of histamine spasm within three minutes. In Table I are shown the mean values obtained by comparing the compounds on the ileum of each of several guinea-pigs. 295C51 is twice as active as 405C49; the latter is equal to mepyramine and twice as active as chlorprophenpyridamine (Chlortrimeton).

TABLE I

RELATIVE ANTIHISTAMINE ACTIVITIES COMPARED WITH THAT OF 405C49 IN ISOLATED GUINEA-PIG ILEUM

Compound	No. of Comparisons	Activity	
		Mean	Limits of Error p=0·95
405C49	—	1·0	—
295C51	6	2·1	1·8–2·4
Mepyramine ..	4	1·1	0·97–1·3
Chlorprophenpyridamine ..	3	0·54	0·42–0·69

Histamine Asthma.—The compounds were compared, using 405C49 as standard, by observing the incidence of "asthma" in groups of guinea-pigs exposed to a histamine aerosol for five minutes, thirty minutes after injecting the antihistamines intraperitoneally. A total of 220 animals received 405C49, and it was estimated that the dose protecting 50% of pigs was 0.094 mg./kg., with limits for p=0.95 of 0.07–0.12 mg./kg. Compound 295C51 was at least five times as active as 405C49, and the mean estimate of the activity ratio for these two compounds was 8.6 (Table II). Mepyramine and chlorprophenpyridamine may be

TABLE II

RELATIVE ANTIHISTAMINE POTENCIES BY THE HISTAMINE ASTHMA TEST IN GUINEA-PIGS AT 30 MINUTES AFTER INTRAPERITONEAL INJECTION AND ONE HOUR AFTER ORAL ADMINISTRATION

Limits of error for p=0·95 in brackets

Compound	Intraperitoneal		Oral	
	No. of Pigs per Compound	Activity	No. of Pigs per Compound	Activity
405C49 ..	220	(1·0)	80	(1·0)
295C51 ..	90	8·6 (5·0–15·0)	80	2·9 (1·5–5·8)
Mepyramine	170	1·5 (1·0–2·1)	80	1·6 (0·79–3·1)
Chlorpropphenpyridamine ..	20	1·8 (0·56–6·1)	80	3·7 (1·8–7·1)

slightly more active than 405C49, but the differences were not significant. No significant differences were detected in the duration of action with doses of the four compounds which showed equal activity at thirty minutes after injection, but there was a tendency for the duration to be shorter with 405C49 than with the other compounds.

All four compounds are less potent when given orally than when given intraperitoneally. For example, the dose of 405C49 preventing asthma in 50% of guinea-pigs exposed to a histamine aerosol one hour after oral administration was 2.3 mg./kg. (limits, p=0.95, 1.4–3.8), i.e., about ten times the intraperitoneal dose. The mean estimates of the relative potencies of the four antihistamines examined (Table II) were not the same for oral administration as for intraperitoneal injection, but the only significant difference was between the 295C51 : 405C49 ratios (p=0.01–0.02). When given by mouth the activities of 295C51 and chlorprophenpyridamine were significantly greater than that of 405C49, and probably greater than that of mepyramine.

Histamine Fall of Blood Pressure.—295C51 and 405C49, like other antihistamines, reduce but do not abolish the depressor action of histamine in the anaesthetized dog. Significant reductions in the histamine effect have been observed with doses as low as 0.01 mg./kg. of 295C49 or 0.05 mg./kg. of 405C49. Our experiments have not been sufficient in number to determine the relative activity of these two compounds, but they indicate that 405C49 may be about as active as mepyramine and that 295C51 may be considerably more active. Very large doses (5 to 10 mg./kg.) not only greatly reduce the histamine response but lower the blood pressure and reduce its response to acetylcholine.

Gastric Secretion.—Wood (1948) observed that mepyramine increased the rate of gastric secretion of cats during a slow infusion of histamine. Using a similar procedure, but taking a continuous record of secretion, we found that 295C51 had the same type of action. A single intravenous dose of 1 mg./kg. was without effect, but after 5 mg./kg. intravenously the rate of secretion doubled in three minutes and reached a maximum in 30 minutes, when its volume was 230% and its total acidity 330% of the pre-injection level. The increased rate of secretion was maintained for over an hour.

Anaphylaxis in the Isolated Ileum

Guinea-pigs were sensitized by intrahepatic injection, at the same time but at different sites, of two volumes of 0.05 ml. of a 10% solution of egg albumen. This procedure, which Barrett (1950) used with horse serum, gave a high incidence of anaphylaxis in guinea-pigs, or strips of smooth muscle from them, when tested with antigen 10 to 16 days later. The action of two or more concentrations of the antihistamines were compared on anaphylactic contracture of strips of isolated guinea-pig ileum (suspended in aerated Tyrode Ringer at 37–39° C.) on the same day using several strips from the same pigs. They were added to the organ bath three minutes before the antigen (egg albumen, 4×10^{-5}). Each anaphylactic contraction was expressed as a percentage of maximal by comparing its extent with that due to a large concentration of histamine (10^{-6}). In the absence of antihistamine the contraction due to the antigen, although taking longer to develop, was usually about maximal; only when this was so was the ileum used for comparing the effect of antihistamines.

The antihistamines greatly retarded the rate of anaphylactic contraction at concentrations much less than those which limited its final extent (Fig. 1). The inhibitory action was proportional to the dose, but usually only very high concentrations (10^{-5} or more) eliminated the response to antigen In Fig. 2 are shown the number of guinea-pigs (in groups of eight) the ileum of which gave a response to antigen of 50% or less of maximal, at each of five concentrations of 405C49, 295C51, and mepyramine. These compounds were approximately equiactive. Very much larger amounts of each were required to limit the response to antigen of the ileum from some animals than in that from others, the range for 50% inhibition lying between 10^{-5} and 3×10^{-7}. These amounts are very much greater than those which are needed to eliminate the spasmogenic action of large concentrations of histamine applied externally to the ileum.

Anaphylaxis in Vivo.—405C49, 295C51, and mepyramine were injected intraperitoneally in doses of 0.4 to 50 mg./kg. into sensitized guinea-pigs, 30 minutes before injecting 0.1 ml. of a 10% solution of egg albumen into the saphenous vein under local anaesthesia (0.1 ml. of 5% procaine). The results are summarized in Fig. 3. When no antihistamine was given nearly all the guinea-pigs developed severe "asthma" within 2–3 minutes, and most of them died in 5–10 minutes. Autopsy revealed typical severe emphysema and in some animals vascular engorgement of the gastrointestinal tract. In contrast, few of the antihistamine-treated animals developed severe asthma or died within 10 minutes. Those animals which succumbed within this time showed a similar postmortem appearance to that of the control animals. Although the antihistamines protected the guinea-pigs to a very large extent against the early mani-

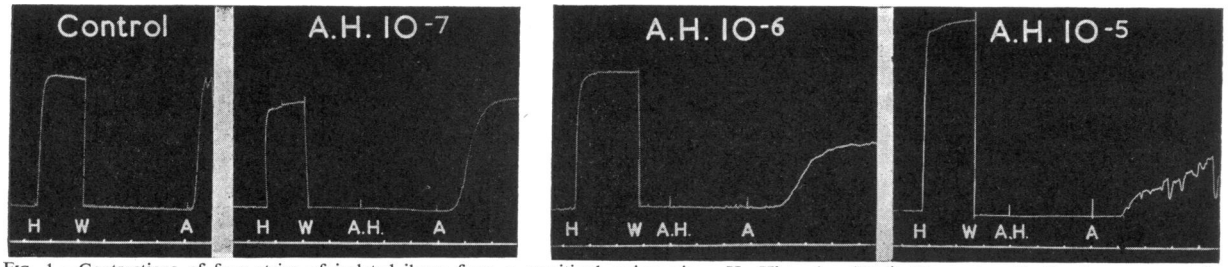


FIG. 1.—Contractions of four strips of isolated ileum from a sensitized guinea-pig. H=Histamine (10^{-6}), W=wash with the drum stopped for 10 minutes, A.H.=antihistamine 295C51 at various concentrations, A=antigen (egg albumen, 4×10^{-5}). Time scale in minutes.

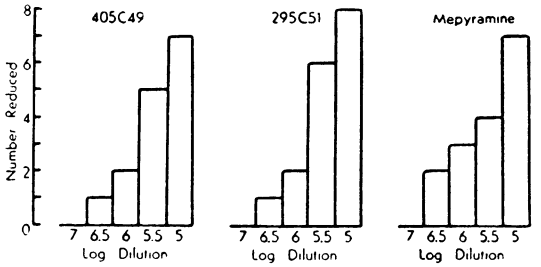


FIG. 2.—Effect of 405C49, 295C51, and mepyramine on the anaphylactic response of isolated guinea-pig ileum. The number of pigs out of eight whose contractures were reduced to 50% or less at various log. dilutions of the antihistamines.

festations of anaphylactic shock, they did not, with the exception of the higher doses of 295C51, prevent later collapse and/or dyspnoea in the majority of animals. At the end of 30 minutes nearly all the animals receiving the higher doses of 295C51 were still alive and mainly in good clinical condition, whereas about half the animals in the 405C49 and mepyramine groups had already died. Twenty-four hours later the mortality was substantially less than that of the controls only in the groups receiving 2 to 50 mg./kg. of 295C51 (during the latter part of this period the single doses of antihistamines given would be exerting little effect). Vascular congestion of the gastro-intestinal system and of other tissues was a more prominent feature than severe emphysema of the lung in the delayed deaths (Fig. 3). It was also found, by sacrificing a number of animals, that vascular engorgement of the intestines was marked in many survivors 30 minutes after administering antigen.

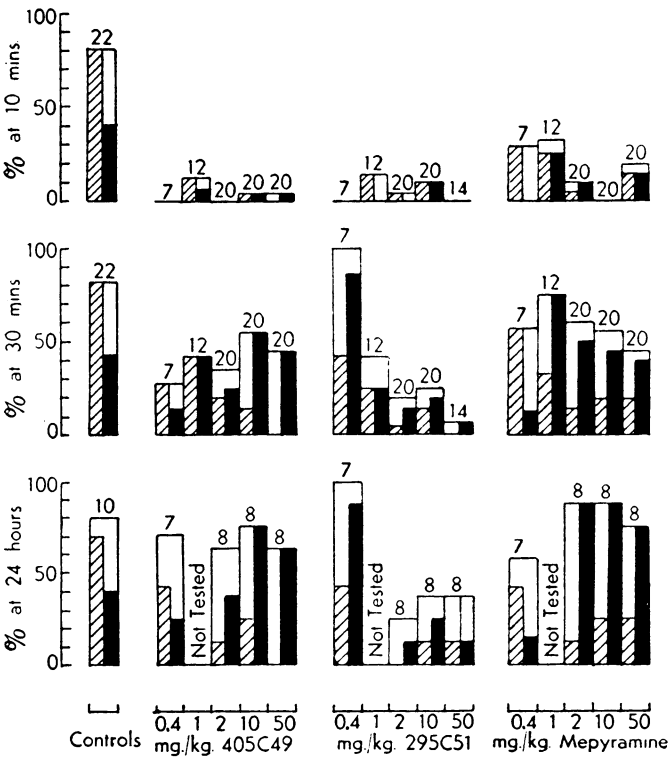


FIG. 3.—The percentage mortality in groups of sensitized guinea-pigs (number used shown above each column) at intervals after testing with antigen (i.v.), preceded by various doses of antihistamines (i.p. 30 minutes previously). The heights of the shaded and black portions show respectively the percentages dying with severe emphysema and with severe vascular congestion of the intestine.

Other Properties

Toxicity.—In mice the LD50s (and limits of error for p=0.95) of 295C51, 405C49, and mepyramine were respectively 21 (18–25), 24 (20–27), and 41 (38–44) mg./kg. intravenously, and 300 (250–360), 180 (160–220), and 170 (160–190) mg./kg. subcutaneously. The LD50 of chlorprophenpyridamine subcutaneously was 260 mg./kg. (limits 210–320).

A dog was unaffected by 5 mg./kg. of 295C51 intravenously, but almost immediately after the injection of a further 10 mg./kg. 15 minutes later, severe tremors, hyperexcitability, and dyspnoea followed by hyperpnoea occurred. These effects disappeared within two hours. The same symptoms followed by severe clonic con-

vulsions were caused by 50 mg./kg. of 405C49 intraperitoneally; the convulsions were completely controlled by pentobarbitone sodium, subsequent recovery from anaesthesia being uneventful. In dogs anaesthetized with pentobarbitone sodium the fatal intravenous doses of 405C49 and 295C51 have varied between 15 and 40 mg./kg. Such amounts caused precipitous falls in blood pressure, apnoea, hyperpnoea, and within 1–2 minutes complete respiratory arrest. There were no tremors or convulsions.

Daily subcutaneous injection of 10 mg./kg. of 405C49 or 295C51 in newly weaned rats, for a period of nine weeks, did not affect their growth, behaviour, erythrocyte counts or macroscopic appearance at autopsy. Dr. D. J. Trevan found no histological evidence of damage due to the drugs in the lungs, livers, spleens, and kidneys of these animals.

Blood Pressure and Breathing.—Large doses of 405C49 and 295C51 lower the blood pressure of cats and dogs under pentobarbitone sodium. In dogs the pressure falls about 10–30 mm. Hg with 2 to 4 mg./kg. and 60 mm. Hg with 10 mg./kg. of 405C49; it falls about 10 to 50 mm. Hg with 1 to 5 mg./kg. and 90 mm. Hg with 30 mg./kg. of 295C51 intravenously. The fall is of short duration with doses below 5 mg./kg., but more persistent with larger amounts. The administration of atropine, or severing both vagosympathetic trunks, did not affect the depressor action of 405C49 in the dog.

Given intravenously in doses of 1 to 10 mg./kg., 405C49 and 295C51 greatly increase the respiratory movements of dogs under pentobarbitone. With the lower doses the effect is small and temporary, but with higher doses, after brief apnoea, the respiratory volume is usually at least trebled for up to half an hour or more.

Action on the Bowel.—The compounds 295C51 and 405C49 had very little effect on the activity of the bowel. Strips of isolated rabbit ileum suspended in aerated Tyrode Ringer at 37–39° C. ceased to contract spontaneously and showed loss of tone when treated with 405C49 and 295C51 at the high concentration of 5×10^{-5}. Concentrations of 10^{-5} caused only slight inhibition, sometimes preceded by a transient increase in motility. 405C49, 20 mg./kg. subcutaneously, did not affect the propulsion of a charcoal meal in rats. Intravenous doses as high as 6 mg./kg. or more of 295C51 reduced the motility of the duodenum and ileum of a dog under pentobarbitone sodium.

Temperature.—The rectal temperatures of resting rabbits fell slightly in some but not all animals, but never more than 1° C., when given 10 mg./kg. of 405C49 or 295C51 intravenously.

Analgesia.—405C49 and 295C51, in doses of 50 mg./kg., had no significant effect on the pain threshold of rats when tested by the pressure method of Green and Young (1951).

Local Anaesthesia.—Both 405C49 and 295C51 caused corneal anaesthesia (to touch with a rounded glass rod) lasting 15 to 45 minutes when applied as 5% solutions, buffered at *p*H 6, to the rabbit eye for one minute. The duration of action was similar to that of mepyramine or chlorprophenpyridamine under the same conditions, when tested in groups of six rabbits.

Atropine-like Properties.—Like mepyramine and chlorprophenpyridamine, 405C49 and 295C51 were remarkably free from atropine-like effects. In mice none of these compounds dilated the pupil except when given in doses nearing the amounts causing fatal respiratory arrest. When tested on isolated guinea-pig ileum the amounts required to reduce spasm due to acetylcholine at a concentration of 5×10^{-7} were approximately a thousand times those which inhibit spasm due to the same concentration of histamine. 405C49, for example, gave 50% relaxation of spasm within three minutes at 7.2×10^{-6} (mean of two tests) when acetylcholine 5×10^{-7} was used, and at 5.6×10^{-9} (mean of 31 tests) against histamine spasm. The estimated activities of 405C49, 295C51, and chlorprophenpyridamine were respectively 0.0006, 0.0015, and 0.0006 times that of atropine sulphate. They reduced the response to acetylcholine in dogs under pentobarbitone sodium only in doses which themselves lowered the blood pressure, i.e., only at very high doses.

Discussion

The antihistamine action of the phenylpyridylallylamines, 405C49 and 295C51, are of the same order of magnitude and as specific as those of mepyramine and chlorprophenpyridamine; the relative potencies of these four compounds in the guinea-pig vary with the method of measurement. 295C51 is considerably more active than the other compounds in relaxing histamine spasm of the guinea-pig ileum *in vitro*. The greater activity is more pronounced when given intraperitoneally in preventing the so-called histamine asthma in the guinea-pig; but when given orally under similar conditions, while its activity is still great, it is now equalled by that of chlorprophenpyridamine

(Table II). In the present experiments intraperitoneal mepyramine is nearly as effective as chlorprophenpyridamine, in agreement with the results of Feinberg, Malkiel, Bernstein, and Hargis (1950). They also found that intraperitoneally the two compounds gave a similar degree of protection against intravenous histamine. In contrast with these findings and the present observation that, when given orally in the asthma test, mepyramine is about half as active as chlorprophenpyridamine, Margolin and Tislow (1950) report that mepyramine given orally has only 5.6% of the activity of chlorprophenpyridamine in protecting against intravenous histamine. The results in guinea-pigs therefore are by no means uniform. For this reason there can be only a broad parallelism between the activities of antihistamines in guinea-pigs and those in man. In reducing the response to intracutaneous histamine in man, 405C49 and chlorprophenpyridamine are about equally potent (405C49 being quicker and shorter acting) (Bain, 1951), while 295C51 is about five times as active (Bain, personal communication). Our results in guinea-pigs are similar. On the other hand, the finding that mepyramine has only 8% of the activity of 405C49 or chlorprophenpyridamine in man (Bain, 1951) contrasts with the results in guinea-pigs described here and by Feinberg, Malkiel, Bernstein, and Hargis (1950), but not with those of Margolin and Tislow (1950).

Dale (1948) distinguishes between the actions of intrinsic and extrinsic histamine. While antihistamines readily antagonize the effects of extrinsic histamine, the action of intrinsic histamine, such as is released during the anaphylaxis of smooth muscle, is more difficult to suppress (Loew, 1947). Many antihistamines have been examined, using several kinds of smooth muscle and different experimental conditions, and it has always been reported that the amount of antihistamine required to inhibit the anaphylactic response is far greater than that which blocks the action of added histamine. In our experiments on the anaphylactic response of isolated guinea-pig ileum to egg albumen it was found that, although the antihistamines (405C49, 295C51, and mepyramine) limited the final extent of anaphylactic contraction only in relatively high concentrations, the amount of antihistamine which caused marked delay in the onset and progress of spasm was comparable with that which blocked the effect of added histamine, and far less than that required to modify the effects of acetylcholine or barium. The height of the anaphylactic contraction was to some extent inversely related to the concentration of antihistamine in contact with the ileum. The concentration of antihistamine required for the same degree of inhibition varied greatly in the different pigs, in some animals 3×10^{-7} being sufficient to halve the contraction. It seems therefore that antihistamines have a very pronounced inhibitory action on the anaphylactic contraction of smooth muscle, and that this inhibition varies in degree with the concentration of antihistamine and the amount of histamine liberated by the antigen-antibody reaction. However, very large concentrations of antihistamine are required completely to eliminate the response to antigen, and these amounts are of the same order as those which inhibit the contraction caused by barium and acetylcholine. This might be due to the high concentration of intrinsic histamine released during the antigen-antibody reaction at the cell membranes. There are, however, a number of experimental findings (Schild, 1936, 1949; Kellaway and Trethewie, 1940; Schild, Hawkins, Mongar, and Herxheimer, 1951) which suggest that the anaphylactic contracture of smooth muscle is not entirely due to histamine release. Kellaway and Trethewie (1940) postulated that a "slow reacting substance," liberated by the antigen-antibody reaction, was partly responsible for the anaphylactic contraction of smooth muscle, and showed that this substance was released more slowly than histamine. This "slow reacting substance" may be responsible for causing the delayed slow contraction of sensitized smooth muscle treated with antigen in the presence of concentrations of antihistamine sufficient to inhibit the rapid histamine contraction.

In the intact animal the antihistamines 405C49, 295C51, and mepyramine are only of limited effectiveness in controlling anaphylactic shock, 295C51 being considerably more effective than the other compounds. All three compounds greatly delay the onset of symptoms and in adequate doses reduce the incidence and severity of asthma and emphysema of the lung, but no single dose has succeeded in eliminating these effects completely. The observations support the generally accepted view that early asthma and emphysema is due to bronchoconstriction caused by liberated histamine, but the later emphysema must be due either to insuperable amounts of intrinsic histamine or to some other factor. As previously reported by Williamson (1936), the main effect in guinea-pigs dying late in anaphylaxis is not emphysema of the lung but congestion and haemorrhage in the gastro-intestinal tract and other tissues. This latter

effect was scarcely influenced by any dose (0.4 to 50 mg. subcutaneously) of mepyramine or 405C49, nor by any but perhaps the highest doses of 295C51. It must therefore be supposed that the vascular component of anaphylaxis is due either to very large amounts of intrinsic histamine (it is known that antihistamines are of limited effectiveness in reducing the fall of blood pressure caused by histamine) or to another factor. Incidentally, it is not possible to produce the severe vascular congestion of the intestine seen in anaphylactic pigs by very large doses of histamine (10 to 1,000 mg./kg. subcutaneously) in either the normal or antihistamine-treated guinea-pig.

Summary

1. *Trans*-l-(4′-chlorophenyl)-l-(2′-pyridyl)-3-pyrrolidinoprop-l-ene maleate (405C49) and *trans*-l-(4′-methylphenyl)-l-(2′-pyridyl)-3-pyrrolidinoprop-l-ene hydrochloride (295C51) show antihistamine actions of the same order of magnitude and as specific as those of mepyramine and chlorprophenpyridamine. The relative activities of these four compounds vary in different tests. In guinea-pigs, 295C51 is easily the most active in relaxing histamine spasm of the ileum *in vitro* and, when given intraperitoneally, in preventing histamine asthma ; given by mouth, its activity is equalled by that of chlorprophenpyridamine.

2. Mepyramine, 405C49, and 295C51 greatly delay the onset and retard the progress of the anaphylactic response of isolated guinea-pig ileum to egg albumen at concentrations comparable to those which block the action of added histamine. Larger concentrations are necessary to limit the final extent of the spasm, the amounts causing 50% inhibition varying in different pigs between wide limits (10^{-5} to 3×10^{-7}). The anaphylactic contraction is eliminated only by very high concentrations, sufficient to antagonize the action of spasmogens other than histamine.

3. 405C49 and 295C51, especially the latter, afford greater protection than mepyramine against anaphylactic shock in the intact guinea-pig, but no single dose has given complete protection. The onset of fatal emphysema of the lung can be greatly delayed by these antihistamines, but it frequently develops later unless large doses are used. The majority of animals die, after considerable delay, whatever the antihistamine treatment, other than large doses of 295C51. Vascular congestion of the intestine and other tissues is then a more marked feature than emphysema. These observations are discussed in relation to the role of histamine and other factors in the anaphylactic response.

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