Brit. J. Pharmacol. (1965), **24**, 725–734.

THE INFLUENCE OF ASCORBIC ACID ON HISTAMINE METABOLISM IN GUINEA-PIGS

BY

W. DAWSON AND G. B. WEST

*From the Department of Pharmacology, School of Pharmacy, University of London, Brunswick Square, W.C.*1

(*Received September* 24, 1964)

The influence of ascorbic acid on anaphylaxis has always been a subject of controversy. For example, Cohen (1939) and van Niekerk (1937) found that ascorbic acid did not protect guinea-pigs from anaphylactic shock, whereas Hochwald (1935) obtained protection with large doses. Further, Giroud, Giroud, Ratsimamanga & Rabinowicz (1936) found that, by using daily doses of ascorbic acid during the period of antibody formation, the degree of sensitization was increased, whilst Zolog (1924) claimed that it was reduced. The present study was designed to re-examine the action of ascorbic acid in anaphylaxis in guinea-pigs and to determine its effect on histamine metabolism and sensitivity. It was also thought of interest to study its effect in anaphylaxis and on the anaphylactoid reaction in rats.

METHODS

Histamine excretion in guinea-pigs. Groups of twelve male guinea-pigs (body weight 400 to 600 g) were given an oral water load (5% of body weight) every 2 hr for 6 hr, and urine was collected in 2-hr samples over 8 hr. To study histamine formation, histidine in doses of 250, 500 or 1,000 mg/kg was either dissolved in the second water load or given intraperitoneally at the same time as the second water load. The urine was assayed directly for its histamine content using the isolated atropinized guinea-pig ileum preparation. The first 2-hr sample represented the basal urinary level of free histamine for each guinea-pig, and subsequent samples gave a measure of the rate of decarboxylation of histidine. The method is similar to that described by Waton (1963).

Sensitivity to histamine aerosol. Groups of twelve male guinea-pigs (body weight 150 to 200 g) were used. Each animal was placed in a desiccator and was made to inhale an aerosol of histamine (1% w/v) which was sprayed into the chamber at a pressure of 10 lb/sq in. As soon as it showed the characteristic convulsive cough, it was removed from the chamber and allowed to breathe air. The time taken to produce the first convulsive cough was called the " collapse time " or " preconvulsion time " and this value was used as a measure of the sensitivity of the animal to the histamine aerosol.

Histamine content. The histamine content of various tissues of guinea-pigs was determined by the method of Parratt & West (1957). Briefly, the tissues were extracted with 10% (w/v) trichloracetic acid (5 ml./g), the excess acid was removed by ether, and the solution was assayed on the atropinized guinea-pig ileum for its histamine content.

Formation of histamine in vitro. The method of Waton (1956) was used to study the rate of decarboxylation of histidine in tissues of guinea-pigs.

Bronchoconstriction in vivo. Guinea-pigs were anaesthetized with chloralose (110 mg/kg, intraperitoneally) and then artificially ventilated with a constant volume pump through a tracheal cannula. Pressure in the trachea was recorded using a transducer system and drugs were injected into the jugular vein.

Anaphylaxis in vivo. Groups of eight male guinea-pigs (body weight 150 to 200 g) were given an intraperitoneal injection of 100 mg of egg albumen. After 3 weeks, the animals were exposed to antigen aerosol (1% w/v, freshly prepared) in a chamber similar to that used for the histamine aerosol. The time taken to produce the first convulsive cough was used as a measure of the sensitivity of the animal to the antigen aerosol, and remained fairly constant over many weeks if the aerosol was given only once a week (Herxheimer, 1952; Smith, 1961).

Anaphylaxis in vitro. Sensitized guinea-pigs were exposed at weekly intervals to antigen aerosol. When consistent collapse times were obtained, the guinea-pigs were killed and their lungs were perfused using the method of Brocklehurst (1960). Antigen was added to the perfusion fluid and the perfusates were assayed for histamine on the atropinized guinea-pig ileum.

Diet deficient in ascorbic acid. Guinea-pigs were made deficient in ascorbic acid by feeding a diet of autoclaved Rank SG1 pellets supplemented with vitamins A, B complex and D. Control animals received the same diet with ascorbic acid (50 mg/guinea-pig/day) included in the drinking water.

Anaphylaxis in rats. Groups of twelve Wistar albino rats were sensitized to horse serum (0.5 ml.) using *Haemophilus pertussis* vaccine as adjuvant. After 10 days, they were challenged intravenously with 1 ml. of the antigen. Other groups of rats were treated intraperitoneally with ascorbic acid or mepyramine before the challenge.

Anaphylactoid reaction in rats. Groups of twelve rats were injected intraperitoneally with dextran (Intradex, 180 mg/kg) and the degree of oedema over 4 hr was noted. Oedema was measured on an arbitrary scale from 0 to 3, and the mean values of each group were expressed as percentages of the maximal possible score. Other rats received either ascorbic acid or L-xylose before, with, or after the dextran. To study the local response, doses of dextran (50 and 200 μg) were injected intradermally into the depilated skin of rats, six injections being given to each rat, each in a volume of 0.1 ml. All animals received intravenous azovan blue dye beforehand (Bonaccorsi & West, 1963). In other experiments, ascorbic acid was mixed with the dextran before intradermal injection.

RESULTS

Guinea-pigs

Excretion of free histamine in urine. The amounts excreted were small but a significant increase ($P<0.01$) occurred after 10 days on the scorbutic diet, as shown in Fig. 1. More histamine was excreted 7 days later when the level was about four-times the control value. After another week on the scorbutic diet, the urinary free-histamine had returned to the control value, and by day 31 the excretion was significantly decreased. The animals died a few days later. The excretion of histamine was nearly doubled in every experiment when aminoguanidine (100 mg/kg) was given intraperitoneally in daily doses. The control value was not changed when ascorbic acid (200 mg/kg) was injected intraperitoneally 15 min before the second water load.

Sensitivity to histamine aerosol. After about 2 weeks on the scorbutic diet, the sensitivity of guinea-pigs to the histamine aerosol was significantly increased ($P<0.01$). This was a transient effect and about 2 weeks later there was a marked decrease in sensitivity which remained until death (Fig. 2). Ascorbic acid (200 mg/kg), given intraperitoneally 15 min before the aerosol, did not protect guinea-pigs, the mean collapse times not differing from the control values.

Sensitivity to antigen aerosol. After 2 weeks on the scorbutic diet, sensitized guinea-pigs showed a decrease in sensitivity to antigen aerosol and by 4 weeks the mean collapse time was more than six-times the control value (Fig. 3,*a*). When sensitization was induced after the animals had been on the scorbutic diet for 3 weeks, the mean collapse time on the first exposure to antigen 3 weeks later was increased more than threefold. The sensi-

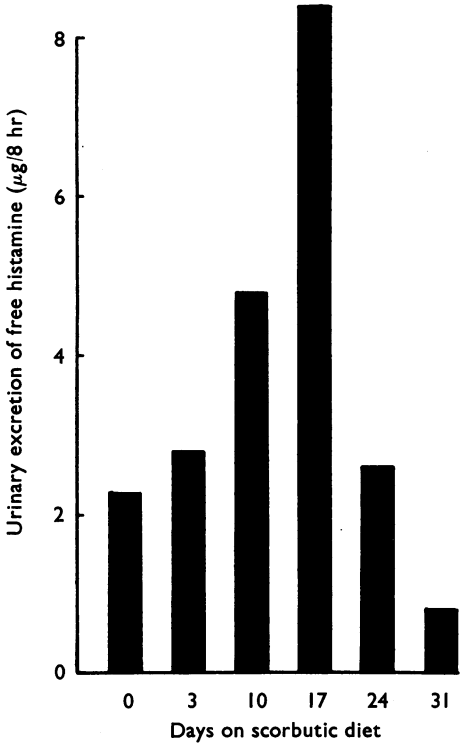

Fig. 1. The effect of feeding guinea-pigs a scorbutic diet for 31 days on the urinary excretion of free histamine (μg/8 hr) after a water load every 2 hr. Note the increased excretion at days 10 and 17, and the marked decrease by day 31. Values are means for groups of twelve animals.

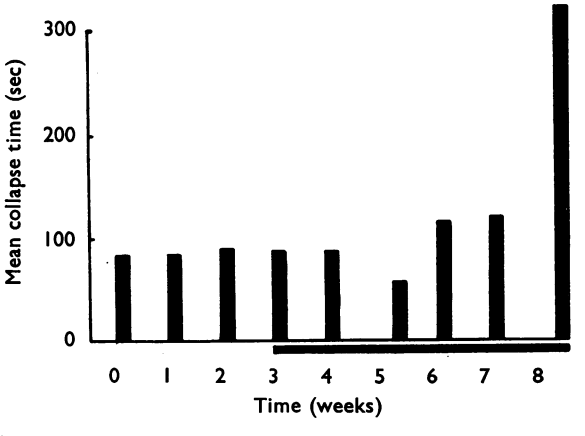

Fig. 2. The effect of feeding guinea-pigs a scorbutic diet for 5 weeks (shown by the horizontal bar) on the mean collapse time (sec) when subjected to a histamine aerosol. Each point is the mean of twelve results. Note the development of insensitivity after 4 weeks on the scorbutic diet.

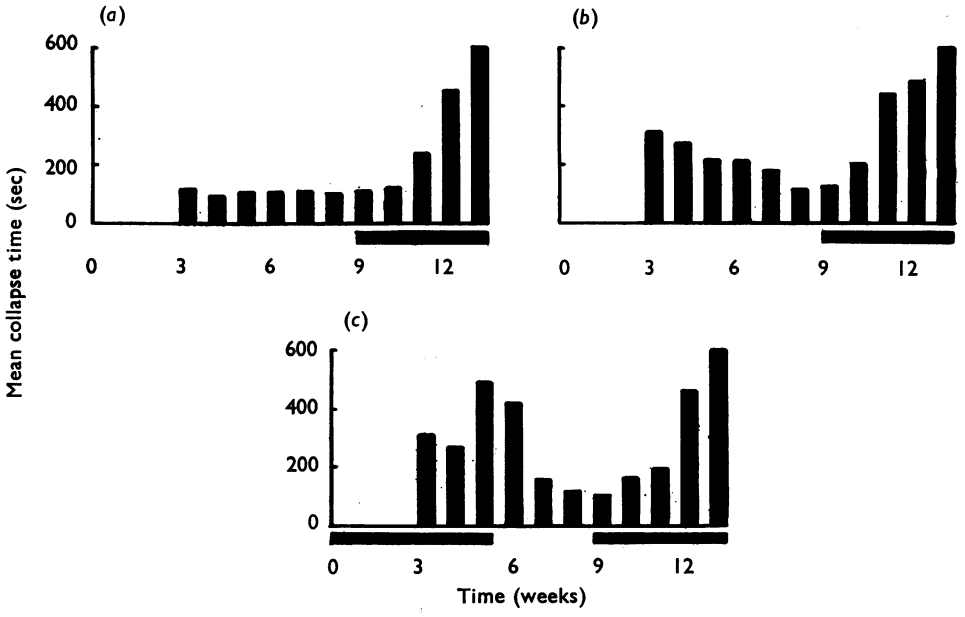

Fig. 3. Mean collapse times (sec) of guinea-pigs subjected at weekly intervals to antigen aerosol. Sensitization to antigen was at zero time. Periods on scorbutic diet are shown by the horizontal bars. Each point is the mean of eight results. (*a*) Complete diet for 9 weeks, followed by scorbutic diet for 4 weeks; (*b*) scorbutic diet for 3 weeks before sensitization, followed by complete diet for 9 weeks and then the scorbutic diet for 4 weeks; (*c*) scorbutic diet for 5 weeks after sensitization, followed by complete diet for 4 weeks and then the scorbutic diet for 4 weeks. Note the development of insensitivity in all cases when ascorbic acid is withdrawn.

tivity to antigen steadily increased over the next 6 weeks at which time ascorbic acid was removed from the diet. The animals rapidly became insensitive and the mean collapse time was more than six-times the control value (Fig. 3,*b*). When sensitization was induced at the same time as the guinea-pigs were placed on the scorbutic diet, the mean collapse time on the first exposure to antigen 3 weeks later was also increased more than threefold. This sensitivity to antigen increased over the next 2 weeks at which time ascorbic acid was restored to the diet. The mean collapse time quickly returned to the control value in the next 4 weeks, after which removal of ascorbic acid from the diet again produced resistance to antigen (Fig. 3,*c*).

In contrast to the result with histamine aerosol, ascorbic acid (200 mg/kg), injected intraperitoneally 15 or 30 min before the antigen aerosol, significantly protected sensitized guinea-pigs fed the complete diet (Fig. 4). Even half this dose was effective 15 min before the aerosol ($P<0.01$). Doses of 100 and 200 mg/kg exerted even greater protection when given intracardially 5 min before the antigen aerosol, mean collapse times being increased more than ten- and fifteen-times respectively.

Histamine release from perfused sensitized lungs. Histamine release by antigen from perfused sensitized lungs was reduced when the guinea-pigs were placed on the scorbutic diet. After 4 weeks, the amount released was halved (Table 1). Ascorbic acid (10 mg/ml. in the perfusion fluid) did not affect the amount released.

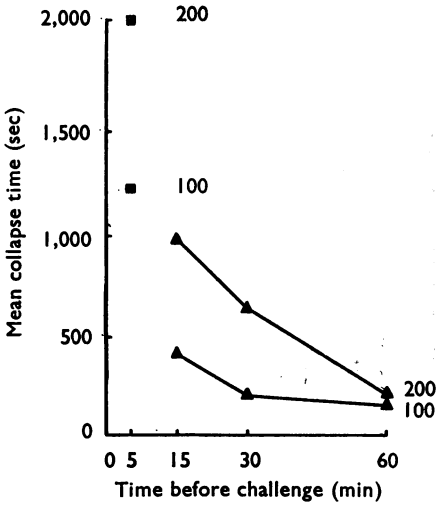

Fig. 4. The effect of ascorbic acid (100 and 200 mg/kg, doses shown) given intraperitoneally (▲——▲) or intracardially (■) at various times before challenge with antigen on the mean collapse times (sec) of guinea-pigs fed the complete diet. Each point is the mean of eight results.

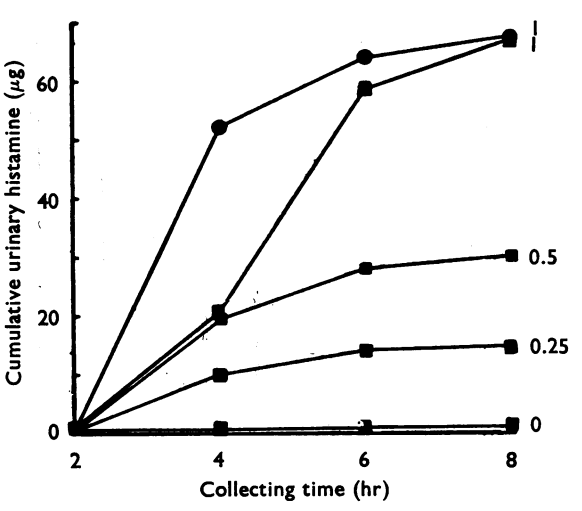

Fig. 5. Dose/response curves for oral histidine (g/kg, doses shown) in guinea-pigs (■——■). Responses are shown as the cumulative urinary free-histamine (μg) excreted over 8 hr. Note that the maximal rate of excretion is at 4 hr for the lower doses but at 6 hr for the highest dose. The urinary excretion of histamine after an intraperitoneal dose of 1 g/kg of histidine (●——●) is also shown. Each point is the mean of eight results.

Histamine content of lungs. Table 1 shows that there was a significant increase in the extractable histamine from the lungs of guinea-pigs after 17 days on the scorbutic diet, but the amount was significantly reduced after 28 days.

Histamine formation in vivo. Dose/response curves using orally administered histidine were first obtained (Fig. 5) by estimating the urinary free-histamine over 8 hr. The maximal rate of excretion with the smaller doses occurred at 4 hr (that is, 2 hr after the histidine) but was delayed to 6 hr with the highest dose. When this high dose was given intraperitoneally, the total amount of histamine excreted over the 8-hr period was similar to that after oral administration but the maximal rate of excretion was brought forward to 4 hr (Fig. 5). Aminoguanidine (100 mg/kg) intraperitoneally produced about a twofold

TABLE 1

EFFECT OF FEEDING A SCORBUTIC DIET TO SENSITIZED GUINEA-PIGS ON THE HISTAMINE CONTENT OF LUNGS, AND ON THE RELEASE OF HISTAMINE FROM THEM BY ANTIGEN

Histamine contents are means and standard errors. Histamine release is as percentage of control values

Days on scorbutic diet	Histamine content (μg/g)	Histamine release (%)
0	31·0±4·2	100
17	42·1±4·0	72
28	21·6±4·8	50

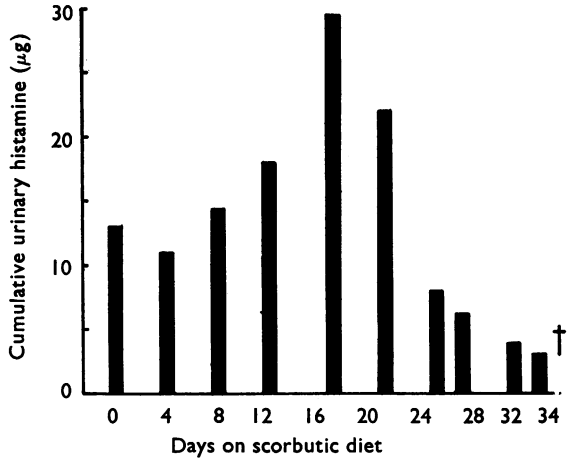

Fig. 6. The effect of feeding guinea-pigs a scorbutic diet for 34 days on the cumulative urinary free-histamine (μg) excreted in 8 hr after an oral histidine load of 250 mg/kg. Each column gives the mean of eight results. Note that the excretion is doubled at 17 days and is very low after 28 days. Death occurred by day 34.

increase in each experiment, irrespective of the dose or the route of injection of histidine.

When guinea-pigs were placed on the scorbutic diet and histidine (250 mg/kg) was administered orally, the total amount of histamine excreted in 8 hr increased to a peak value at day 17 and then dropped sharply to reach very low levels just before death by day 34 (Fig. 6). Animals at 17 days on the scorbutic diet also excreted about twice as much hist-

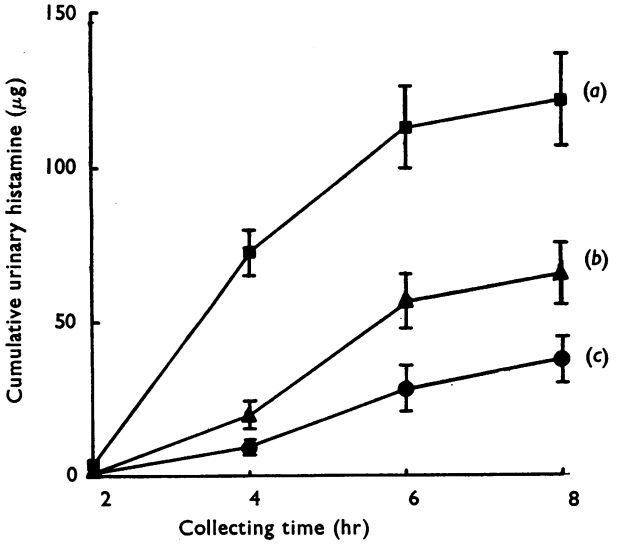

Fig. 7. The effect of an oral histidine load (1 g/kg) on the cumulative urinary excretion of histamine (μg in 8 hr) by guinea-pigs (*a*) after 17 days on a scorbutic diet, (*b*) on a complete diet, and (*c*) on a complete diet but after an intraperitoneal dose of ascorbic acid (200 mg/kg) 15 min before the histidine. Each value is the mean of eight results, with standard errors shown by the vertical lines.

amine as did the control guinea-pigs when the histidine dose was increased to 1 g/kg and the maximal excretion was brought forward to 4 hr (Fig. 7,*a* and *b*). Ascorbic acid (200 mg/kg), given intraperitoneally 15 min before the histidine, halved the histamine excretion values when 1 g/kg of histidine was used (Fig. 7,*c*). The urinary free-histamine excretion over 8 hr was not increased by ascorbic acid *per se* or by orally administered histamine (10 mg/kg).

Histamine formation in vitro. The histidine decarboxylase activities of liver and kidney from guinea-pigs which had been 17 and 28 days on a scorbutic diet were similar to those of tissues from control animals. Ascorbic acid in concentrations up to 10 mg/ml. in the incubation mixture also did not alter these activities.

Sensitivity to intravenous histamine. Intravenous histamine (5 μg) produced a marked bronchoconstriction in guinea-pigs as indicated by an increase in tracheal pressure with constant volume lung inflations, and this was prevented by previously injecting ascorbic acid (500 mg/kg) intravenously within 10 min of the histamine injection. This dose of ascorbic acid, when given intraperitoneally, markedly reduced the histamine response, as did smaller doses (for example, 100 and 200 mg/kg) injected intravenously.

Reserpine (four daily doses of 2 mg/kg, intraperitoneally), pempidine (8 mg/kg, intravenously) and pronethalol (10 mg/kg, intravenously) did not modify the action of ascorbic acid in reducing the histamine response.

Rats

Ascorbic acid and anaphylaxis. Ascorbic acid (200 mg/kg) slightly reduced the mortality rate of rats undergoing anaphylactic shock, but doses of mepyramine as high as 10 mg/kg were ineffective. When, however, mepyramine was given as well as ascorbic acid, complete protection was achieved (Table 2). A lower dose of ascorbic acid (100 mg/kg) was ineffective, even with the higher dose of mepyramine.

TABLE 2

EFFECT OF ASCORBIC ACID AND MEPYRAMINE ON THE MORTALITY RATE OF RATS UNDERGOING ANAPHYLACTIC SHOCK

There were twelve rats per group, sensitized to horse serum. Note that complete protection is obtained when large doses of both drugs are used together

Group	Ascorbic acid (mg/kg)	Mepyramine (mg/kg)	Mortality (%)
1	0	0	100
2	0	1	100
3	0	10	100
4	200	0	67
5	200	1	33
6	200	10	0

Ascorbic acid and the anaphylactoid reaction. The anaphylactoid reaction due to dextran developed at a slower rate when a single dose of ascorbic acid (1.5 g/kg) was injected intraperitoneally together with the dextran. When two similar doses of ascorbic acid were injected together with and 30 min after the dextran, the reaction was considerably delayed in onset and suppressed in magnitude. When three doses of ascorbic acid were used at 30-min intervals, an even greater reduction in response was obtained (Fig. 8). Three doses of L-xylose, a breakdown product of ascorbic acid, when given at 30-min intervals, likewise

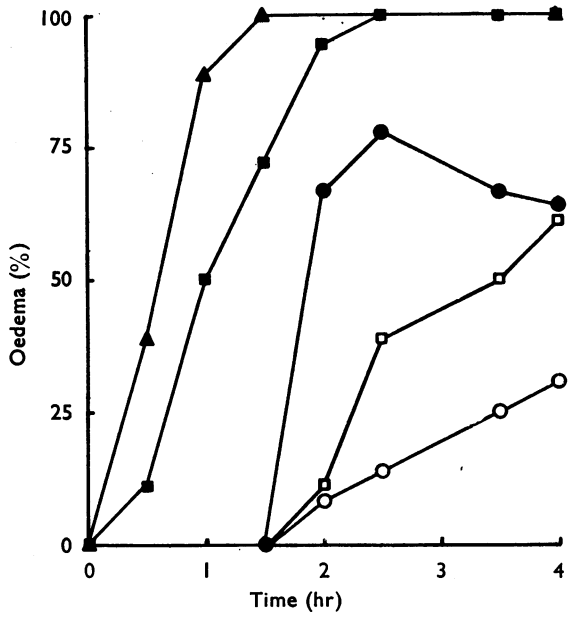

Fig. 8. The effect of one (■——■), two (□——□) and three (○——○) doses of ascorbic acid (1.5 g/kg) on the anaphylactoid reaction to dextran in rats, measured on a shock-score system (see Methods). Control values (▲——▲) without ascorbic acid are also recorded. Note that the reaction is delayed in onset by using more than one dose of ascorbic acid. The effect of three doses of L-xylose (1.5 g/kg) is also shown (●——●).

delayed the onset and reduced the dextran reaction but they were less effective than three doses of ascorbic acid.

When dextran (50 and 200 μg) was given intradermally, the increase in vascular permeability was reduced by the simultaneous injection of ascorbic acid (1 mg).

DISCUSSION

The results show that, during the initial phases of ascorbic-acid deficiency in the guinea-pig, (1) the basal urinary excretion of histamine is considerably increased, (2) the amount of histamine formed and excreted in the urine after an oral load of histidine is increased, (3) the histamine content of the lungs is raised and (4) the sensitivity to histamine aerosol is slightly increased. Thus, 14 to 17 days after changing to the diet deficient in ascorbic acid, the basal urinary values of histamine are raised fourfold and the conversion of histidine to histamine is doubled when the urinary levels of histamine are examined. Administration of ascorbic acid before the histidine load considerably decreases the conversion although it does not alter the basal urinary excretion value and does not influence the histidine decarboxylase activity in tissues when estimated by an *in vitro* method. The increase in sensitivity to a histamine aerosol at this time may be linked with the lowered level of circulating corticosteroids, as reported by Prunty, Clayton & Hammant, 1957.

As the scorbutic state develops further, the basal urinary excretion of histamine is considerably reduced, as also is the amount of histamine formed and excreted after an oral

load of histidine; in addition, the histamine content of the lungs is lowered and the sensitivity of the animal to the histamine aerosol is decreased. Thus, after 30 days on the scorbutic diet, there is less histidine converted to histamine and the guinea-pig becomes very insensitive to the histamine aerosol. At this stage, when clinical signs of scurvy are prominent, the raised levels of circulating corticosteroids (Prunty *et al.*, 1957) may also be responsible for this insensitivity to histamine. In fact, we have recently found that hydrocortisone in doses of 25 mg/kg increases the mean collapse times of control guinea-pigs subjected to the histamine aerosol whilst ascorbic acid, in doses up to 200 mg/kg, is without effect.

When sensitized guinea-pigs are subjected to the antigen challenge whilst on a diet deficient in ascorbic acid for 14 to 17 days, there is no increase in sensitivity. Instead, at this time when the histamine sensitivity is increased, the animals are already less sensitive to the antigen aerosol and become much less sensitive as time progresses. When sensitization is induced in the scorbutic state, sensitivity to antigen is decreased but is restored to normal by incorporating ascorbic acid in the drinking water. Similarly, when the scorbutic diet replaces the complete diet during the period of sensitization, there is also a decrease in sensitivity to antigen which is restored by ascorbic acid. In all these cases, the subsequent removal of ascorbic acid from the diet results in a quick onset of insensitivity to antigen. In fact, the release of histamine from guinea-pig lungs *in vitro* by the antigen is markedly reduced when the animals are maintained on the scorbutic diet. The results also show that administration of ascorbic acid either intraperitoneally or intracardially to guinea-pigs maintained on the complete diet produces insensitivity to antigen when the acid is given a short time before the challenge. It is interesting to note that the mean collapse times of guinea-pigs subjected to antigen aerosol, when doses of mepyramine are used to inhibit the reaction, are increased no more than eight-times (Smith, 1961), whereas with only moderate doses of ascorbic acid these times are increased more than fifteen-times. Moreover, intravenous ascorbic acid protects the guinea-pig against the bronchoconstriction produced by intravenous histamine.

When histidine is administered to control guinea-pigs by the oral and intraperitoneal routes, the same total amount of free urinary histamine is found. This result confirms that decarboxylation of histidine in the gut does not form an important source of histamine in the guinea-pig (Waton, 1963). Aminoguanidine, an inhibitor of histaminase, doubles the output of free histamine in the urine of control guinea-pigs and has a similar effect on the conversion of histidine to histamine when the amino acid is given orally or intraperitoneally. In scorbutic animals, aminoguanidine also doubles the urinary output of free histamine. Thus, histaminase is probably unaffected by the withdrawal of ascorbic acid from the diet, and we have recently found that the histaminase activity of guinea-pig tissues *in vitro* is also unaffected by the presence of ascorbic acid.

In rats undergoing anaphylaxis, ascorbic acid exerts a slight protection which is grossly enhanced by the simultaneous administration of otherwise non-effective doses of mepyramine. This synergism has not so far been found in guinea-pigs. Ascorbic acid also antagonized the anaphylactoid reaction due to dextran in rats when given in large divided doses, and so does xylose, one of its breakdown products. However, mepyramine which is non-effective in this test does not enhance the action of ascorbic acid in preventing the dextran reaction.

SUMMARY

1. The influence of ascorbic acid on anaphylaxis and histamine metabolism in guinea-pigs has been studied.

2. On a scorbutic diet, there is first an increased formation of histamine, as shown by an increased urinary output of free histamine after an oral load of histidine; later, in terminal scurvy, the formation is greatly reduced.

3. Sensitivity to antigen aerosol is decreased from about the second week on the ascorbic acid-deficient diet and the animals become completely insensitive by the fourth week, whereas sensitivity to histamine aerosol is considerably decreased only after 4 weeks on the scorbutic diet.

4. Ascorbic acid, when given immediately before the antigen challenge, protects guinea-pigs from anaphylactic shock but has no effect against the convulsions produced by histamine aerosols.

5. Decarboxylation of histidine occurs chiefly in the tissues of guinea-pigs and is affected by variations in the ascorbic acid content of the diet whereas histaminase activity is not modified.

6. For complete sensitization of guinea-pigs to antigen, ascorbic acid appears to be essential; it is also necessary for the maintenance of the sensitized state.

7. Ascorbic acid, together with large doses of mepyramine, completely protects rats from anaphylactic shock, yet large divided doses of ascorbic acid alone prevent the anaphylactoid reaction to dextran.

This work was supported by research grants from the Pharmaceutical Society of Great Britain and the Medical Research Council (to W. D.).

REFERENCES

BONACCORSI, A. & WEST, G. B. (1963). Absence of capillary permeability response in rats to dextran and egg-white. *J. Pharm. Pharmacol.*, **15**, 372–378.

BROCKLEHURST, W. E. (1960). The release of histamine and formation of slow reacting substance (SRS-A) during anaphylactic shock. *J. Physiol. (Lond.)*, **151**, 416–435.

COHEN, M. B. (1939). Vitamin C deficiency: sensitivity to neoarsphenamine and anaphylactic shock. *J. Allergy*, **10**, 15–26.

GIROUD, A., GIROUD, P., RATSIMAMANGA, R. & RABINOWICZ, M. (1936). Pouvoir antianaphylactique de l'acide ascorbique chez le cobaye; importance de l'alimentation et du taux de l'acide ascorbique sur la sensibilité de l'organisme. *C.R. Soc. Biol. (Paris)*, **123**, 1146–1148.

HERXHEIMER, H. (1952). Repeatable "microshocks" of constant strength in guinea-pig anaphylaxis. *J. Physiol. (Lond.)*, **117**, 251–255.

HOCHWALD, A. (1935). Anaphylaktischer Shock und Vitamin C. *Z. ges. exp. Med.*, **97**, 433–439.

PARRATT, J. R. & WEST, G. B. (1957). Release of 5-hydroxytryptamine and histamine from tissues of the rat. *J. Physiol. (Lond.)*, **137**, 179–189.

PRUNTY, F. T. G., CLAYTON, B. E. & HAMMANT, J. E. (1957). Experiments on the level of blood corticotrophin with particular reference to scurvy. *Ciba Found. Colloquia on Endocrinology*, **11**, 150–160.

SMITH, W. G. (1961). Anti-anaphylactic activity of ethanolamine and choline. *J. Pharm. Pharmacol.*, **13**, 1–11.

VAN NIEKERK, J. (1937). Anaphylaxis and Vitamin C. *J. Allergy*, **8**, 446–449.

WATON, N. G. (1956). Studies on mammalian histidine decarboxylase. *Brit. J. Pharmacol.*, **11**, 119–127.

WATON, N. G. (1963). Urinary excretion of free histamine in guinea-pigs after oral histidine. *J. Physiol. (Lond.)*, **165**, 174–178.

ZOLOG, M. (1924). Action de l'absence de Vitamine C sur l'anaphylaxie. *C.R. Soc. Biol. (Paris)*, **91**, 215–217.