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EFFECT OF ASCORBIC ACID ON HISTAMINE METABOLISM IN SCORBUTIC GUINEA-PIGS

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SUMMARY

1. In guinea-pigs fed an ascorbic-acid-free diet, as the ascorbic acid levels decreased the histamine levels in blood and urine rose steadily to maxima in about 10–12 days. The elevated histamine levels persisted in the blood and urine of scorbutic guinea-pigs and the histamine levels in lung, gastric mucosa and spleen also increased.

The increased histamine content of the urine, blood and other tissues in the ascorbic-acid-depleted condition could be brought back to normal levels by administration of a single dose of ascorbic acid 5 mg/100 g body wt. guinea-pig.

3. The drop in the elevated histamine level was not due to an indirect effect of ascorbic acid on histamine forming capacity, histaminase activity or histamine release.

INTRODUCTION

Previously we reported that a function of ascorbic acid was the detoxification of histamine (Subramanian, Nandi, Majumder & Chatterjee 1973). We observed that under a variety of stress conditions, namely administration of drugs, vaccines, toxoids and physical stress, the formation or release of histamine was increased in the rat and guinea-pig, and that ascorbic acid administration resulted in detoxification of histamine in the body (Subramanian, Nandi, Majumder & Chatterjee, 1974; Nandi, Subramanian, Majunder & Chatterjee, 1974). We considered that if the effect of ascorbic acid in detoxifying histamine was a physiological function of the vitamin, then during ascorbic acid deficiency, ascorbic acid would not be available for detoxification of histamine and as a result the histamine levels in the blood, other tissues and urine would increase. To test this hypothesis, we have investigated the effect of ascorbic acid on histamine metabolism in scorbutic guinea-pigs and the results are presented in this communication.

METHODS

Male short-hair guinea-pigs, of average body weight 200 ± 10 g, were fed the ascorbic-acid-free diet as described before (Subramanian *et al.* 1974). Control guinea-pigs were given the ascorbic-acid-free diet plus a daily oral dose of ascorbic acid, 1 mg/100 g body wt.

Urinary and tissue ascorbic acid levels were determined by titration (Chatterjee, 1970). Urinary and tissue histamine, histamine-forming capacity and plasma histaminase were estimated by a spectrofluorimetric method using *O*-phthalaldehyde as described previously (Subramanian *et al.* 1974). Blood histamine content was determined in trichloroacetic acid filtrate following the bio-assay technique described by Parratt & West (1957). Similar results were obtained using the fluorimetric method. The release of histamine from peritoneal mast cells was measured by the method of Bloom & Hagermark (1965) except that ascorbic acid (7 μmole) was added to the incubation system when needed, and from lung tissue by the method of Bouhuys & Lindell (1961), with addition of 2 μmole ascorbic acid to the incubation medium when required.

The basophil leucocytes from twelve guinea-pigs were counted using the method of Cooper & Cruickshank (1966). The peritoneal mast cell count was made by injecting each guinea-pig I.P. with 10 ml. solution containing: 5 ml. Tyrode (Bloom & Hagermark, 1965); 0·5 ml. 1% EDTA; 2 ml. 0·5% cetyl pyridinium chloride (capacol) and 2·5 ml. toluidine blue solution (Cooper & Cruickshank, 1966). The peritoneal cells were isolated with the method of Bloom & Hagermark (1965), dispersed in 0·5 ml. 0·1% EDTA followed by 0·5 ml. toluidine blue solution and counted in the same way as the basophil leucocytes.

RESULTS

When guinea-pigs were fed a diet free from ascorbic acid we observed that as the blood ascorbic acid content began to fall the histamine level in the blood started to rise steadily, reaching a maximum in about 11 days (Fig. 1). In control guinea-pigs fed an ascorbic-acid-free diet plus a daily oral dose of ascorbic acid, 1 mg/100 g body wt., the blood ascorbic acid level did not decrease and blood histamine level did not increase within the experimental period. To nullify the effect of inanition on histamine metabolism (Petillo, Gulbenkian & Tabachnick, 1969), the control guinea-pigs were pair-fed with the scorbutic guinea-pigs: the food consumption (mean $\pm$ S.D. of observation) was 12 ± 2 g in the first week, 9 ± 2 g in the second week and 6 ± 1 g in the third week.

The decrease in ascorbic acid levels in the blood followed by an increase in the histamine level was also found in the urinary excretion (Fig. 2). The rise in the level of histamine in the urine of guinea-pigs in the scorbutic condition has been shown by Dawson & West (1965) after animals were fed a high dose of histidine followed by a water load. Table 1 shows that in ascorbic acid depletion, histamine levels in different tissues namely the lung, gastric mucosa and spleen, are also elevated.

When the moderate dose of ascorbic acid needed to maintain the

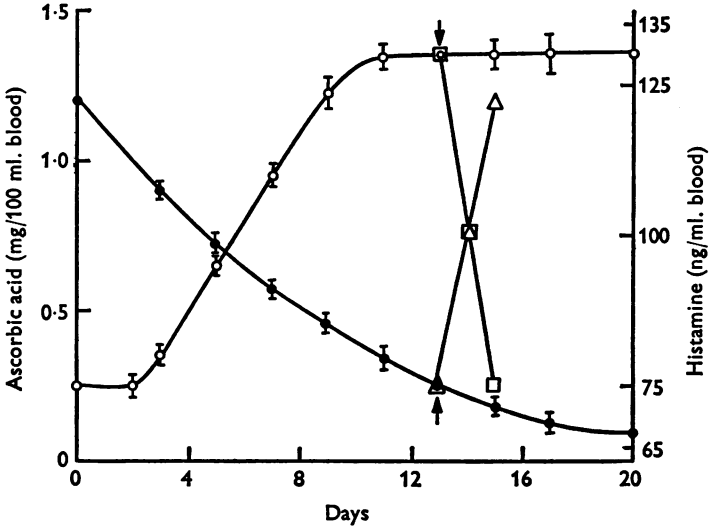

Fig. 1. Ascorbic acid and histamine levels in blood from guinea-pigs fed an ascorbic-acid-free diet. ●—●, ascorbic acid; ○—○, histamine. ↑, ↓, administration of a single dose of ascorbic acid 5 mg/100 g body wt. guinea-pig; △—△, □—□, subsequent ascorbic acid and histamine levels.

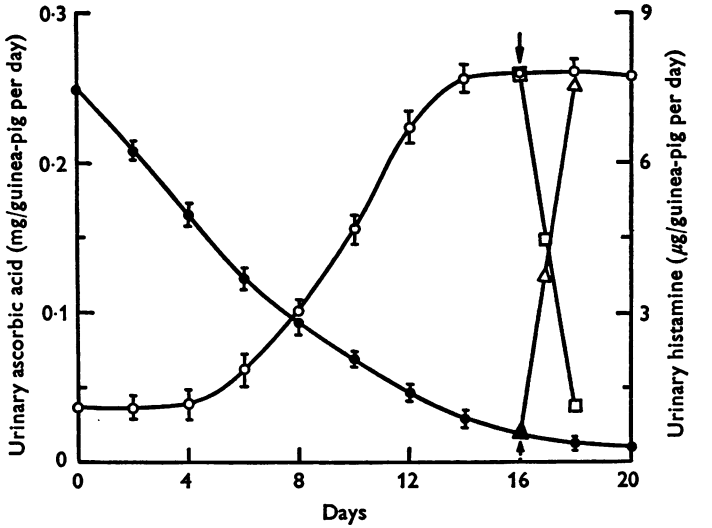

Fig. 2. Ascorbic acid and histamine levels in urine from guinea-pigs fed an ascorbic-acid-free diet. Symbols and ↑, ↓, as in Fig. 1. In control guinea-pigs fed an ascorbic-acid-free diet plus a daily oral dose of ascorbic acid 1 mg/100 g guinea-pig, the basal value of ascorbic acid and histamine in urine did not change within the experimental period.

guinea-pigs, 1 mg/100 g body wt. per day (Goldsmith, 1961), was given with the ascorbic-acid-free diet we found that the blood and urinary ascorbic acid contents did not diminish and the histamine levels in blood, urine, lung, gastric mucosa and spleen did not increase (see Figs. 1 and 2 and Table 1). A dose of ascorbic acid of less than 1 mg/100 g body wt. per day was less effective.

TABLE 1. Effect of ascorbic acid on tissue histamine levels in guinea-pigs fed an ascorbic-acid-free diet

		Histamine content (μg/g tissue)		
	Treatment	Lung	Gastric mucosa	Spleen
Control guinea-pigs	Ascorbic acid 1 mg/100 g body wt per day for 16 days	24·1 ± 2·6	4·1 ± 0·3	2·0 ± 0·1
Guinea-pigs fed an ascorbic-acid-free diet				
Initial value	—	23·9 ± 2·5	4·0 ± 0·2	1·9 ± 0·1
Values obtained on the 17th day	None	45·2 ± 3·0	10·0 ± 0·4	7·5 ± 0·3
Values obtained on the 17th day	A single oral dose of 10 mg ascorbic acid on the 16th day	26·4 ± 1·8	4·8 ± 0·3	2·2 ± 0·1
Values obtained on the 17th day	A single oral dose of 10 mg 2,6-dichlorophenolindophenol on the 16th day	46·1 ± 2·7	9·9 ± 0·4	7·6 ± 0·4
Values obtained on the 17th day	A single oral dose of of 10 mg cysteine on the 16th day	45·0 ± 2·9	10·0 ± 0·3	7·4 ± 0·3

The experimental details are given in the Method section. The body wt. of guinea-pigs was 200 ± 10 g.

The histamine values given represent those obtained on the seventeenth day. Each value given is the mean of twelve observations ± S.E. of mean. Tissues from a pair of guinea-pigs were pooled for two separate observations.

Control guinea-pigs were pair-fed with the scorbutic guinea-pigs.

The increase in histamine levels in the urine, blood and other tissues of ascorbic-acid-depleted guinea-pigs were probably not due to an enhancement of histamine-forming capacity or an inhibition of histaminase (EC 1436) activity (Franzen & Eysell, 1969). The histamine-forming capacity of gastric mucosa (histamine formed 9·5 ± 0·5 ng/mg protein per 90 min) and plasma histaminase (5·9 ± 0·2 Im.U./100 ml. plasma) remained unaltered in guinea-pigs fed an ascorbic-acid-free diet for 17 days.

Also, the raised blood and tissue histamine levels were not due to an increase in basophil leucocytes and tissue mast cells. The basophil leucocytes, 50 ± 10 mm^{-3}, and peritoneal mast cell counts, 40 ± 10 mm^{-3}, did not increase in guinea-pigs fed the ascorbic-acid-free diet for 17 days. Thus, it would appear that the increased body histamine level in the scorbutic condition was not due to an indirect effect of ascorbic acid depletion.

TABLE 2. Effect of ascorbic acid on histamine release from peritoneal mast cells

Additions	Cells	Supernatant	Total	Release of histamine (%)
	Rats, histamine (μg)			
None	41·80	1·09	42·89	2·5
Ascorbic acid	40·00	0·94	40·94	2·2
Compound 48/80	17·10	23·89	40·99	58
Compound 48/80 + ascorbic acid	16·01	23·47	39·48	58
	Control guinea-pigs, histamine (ng)			
None	272	*	272	—
Ascorbic acid	264	*	264	—
Compound 48/80	52	210	262	80
Compound 48/80 + ascorbic acid	58	208	266	80
	Ascorbic-acid-depleted guinea-pigs, histamine (ng)			
None	448	*	448	—
Ascorbic acid	452	*	452	—
Compound 48/80	72	368	440	81
Compound 48/80 + ascorbic acid	64	362	426	80

Peritoneal mast cells from four rats or guinea-pigs were isolated following the method of Bloom & Hagermark (1965) and divided into four equal portions. For other experimental conditions see Methods section.

Ascorbic acid, 7 μmole. Compound 48/80, 2·5 μg, is a condensation product of *N*-methyl *p*-methoxy-phenethylamine with formaldehyde (Sigma Chemical Company, U.S.A.).

Ascorbic-acid-depleted guinea-pigs were fed an ascorbic-acid-free diet and killed on 17th day.

* Trace which could not be estimated.

The increased blood and urinary histamine in the ascorbic-acid-depleted condition could be brought back to the normal level by administration of a single dose of ascorbic acid, 5 mg/100 g body wt. (Figs. 1, 2). Under such conditions the increased tissue histamine contents also dropped almost to the normal level (Table 1). A dose of ascorbic acid smaller than 5 mg/100 g body wt. was less effective. Ascorbic acid could not be replaced by other reducing agents such as 2,6-dichlorophenolindophenol or cysteine (Table 1).

The drop in the elevated histamine levels in the urine and tissues after a single administration of ascorbic acid was not owing to (i) decreased histamine-forming capacity, (ii) increased histaminase, or (iii) inhibition of histamine release. The histamine-forming capacity of gastric mucosa and plasma histaminase activity, as mentioned above, were not affected by ascorbic acid administration. Similar observations were made in the rat (Subramanian *et al.* 1974). It was also seen that ascorbic acid has no effect on the release of histamine from mast cells (Table 2) or lung tissue. The histamine contents of lung (μg/g tisue) was: in the rat, $9{\cdot}1 \pm 0{\cdot}34$; control guinea-pig, $24{\cdot}2 \pm 1{\cdot}1$; and ascorbic acid-depleted guinea-pig, $46{\cdot}2 \pm 1{\cdot}2$. The addition of Compound 48/80 (3 mg) resulted in 8·9 % release of histamine in the rat and 13 % in guinea-pig not affected by the addition of ascorbic acid (2 μmole). Conditions used to determine histamine release from lung tissue were similar to those described by Bouhuys & Lindell (1961).

TABLE 3. Ascorbic-acid-mediated histamine disappearance from post-mitochondrial fraction of guinea-pig liver

Condition	Ascorbic acid content (μg)		Histamine content (μg) Initial*	Histamine content (μg) After incubation
Control guinea-pigs	Initial	200	7·45	1·51
	After 30 min aeration	20	7·45	7·31
	After 30 min aeration followed by addition of 176 μg ascorbic acid	196	7·45	1·78
Ascorbic-acid-depleted guinea-pigs†	Initial	18	7·9	7·87
	After addition of 176 μg ascorbic acid	194	7·9	2·01

* This includes the histamine content of liver (2·05 μg/g liver in the control guinea-pigs and 2·5 μg/g liver in the ascorbic-acid-depleted guinea-pigs) plus 6 μg histamine added to the system. The recovery of histamine was 90 %.

† Guinea-pigs were fed the ascorbic-acid-free diet for 18 days and the experiment performed on the 19th day. The control guinea-pigs received a daily oral supplementation of ascorbic acid 1 mg/100 g body wt.

The detoxification of histamine in the body by ascorbic acid through activation of *N*-methyl transferase (EC. 2.1.1.8, Franzen & Eysell, 1969) is yet to be shown. However, the effect of ascorbic acid on histamine break-down could be demonstrated at the subcellular level.

Livers from four guinea-pigs were homogenized in 3 vol. 0·05 M sodium phosphate buffer, pH 7·2, and centrifuged at 10,000 *g* for 20 min at 2–4° C. The supernatant, the post-mitochondrial fraction, was used for the experiments: each incubation flask contained 4 ml. supernatant in 0·05 M sodium phosphate buffer, pH 7·2. The final

volume was 5 ml. Histamine, 6 μg, was added to each incubation flask and the incubation was carried out in a Dubnoff-type shaker for 90 min at 37° C. Ascorbic acid was estimated by titration and histamine by a spectrofluorimetric technique as stated in the Methods section.

When the liver post-mitochondrial fraction from control guinea-pigs fed ascorbic acid (1 mg/100 g body wt. per day) was incubated with histamine 80% of the histamine disappeared (Table 3). However, after the post-mitochondrial fraction was aerated to oxidize the ascorbic acid and then incubated with histamine, the added histamine could be recovered to an extent of 98%. When ascorbic acid was added to the aerated tissue, to make up the original content, the histamine again disappeared. Table 3 further shows that the post-mitochondrial fraction from ascorbic-acid-depleted guinea-pigs having a very low ascorbic acid content could not destroy the added histamine. Again, if ascorbic acid was added, to bring its concentration back to the normal level, the added histamine underwent break-down.

DISCUSSION

The average daily requirement of the guinea-pig for ascorbic acid is about 1 mg/100 g body wt. (Goldsmith, 1961). With such an intake of the vitamin, the body histamine level remained at the basal value. However, when the guinea-pigs were fed an ascorbic-acid-free diet, the blood histamine level increased steadily long before the onset of scurvy, and this was reflected in increased histamine in the urine and tissues. The histamine level rose to a maximum in about 10–12 days and this elevated level persisted in the scorbutic guinea-pig. Histamine is normally produced in the body and it has a physiological function. But an elevated histamine level is pathological (Kahlson & Rosengren, 1968). However, increased histamine content of the blood and other tissues in the scorbutic condition could be brought back to normal by the administration of a single dose of ascorbic acid 5 mg/100 g body wt. of guinea-pig. The drop in the elevated histamine level was not due to an indirect effect of the vitamin. Using a model system, we have recently shown that ascorbic-acid-mediated histamine detoxification takes place through break-down of the imidazole nucleus and histamine is ultimately converted to aspartic acid (Chatterjee, Majunder, Nandi & Subramanian 1975).

Scurvy is regarded as a typical haemorrhagic disease. One of the chief lesions in scurvy is swelling and degeneration of the capillary endothelium accompanied by leakage of blood through capillaries. Incidentally, histamine has a very powerful vasodilating action on minute blood vessels, particularly the capillaries, and a high histamine level would lead to hyperaemia and increased capillary permeability. This shows that in

ascorbic acid depletion there is probably a correlation between the high and persistent endogenous histamine level and appearance of the characteristic scurvy symptom of capillary degeneration. Scurvy could not be produced by exogenous histamine. This was apparent, because a high blood histamine level could not be maintained by histamine injection in guinea-pigs. An increase in the blood histamine level from 75 to only 105 ng/ml. could be obtained 1 hr after I.P. injection of 250 μg histamine but the value came down to normal in about 3 hr.

Scurvy comes before an animal with ascorbic acid depletion dies. There is a wide gap between full health and a scorbutic condition (Szent-Györgyi, 1974). The transition between optimum nutrition and frank deficiency is gradual and the minor degrees of deficiency without clinical manifestations are difficult to detect. Although clinical scurvy is a rare disease in most of the developed countries, there is undoubtedly a great deal of subclinical ascorbic acid deficiency. At present there is no satisfactory method of identifying subclinical scurvy. If the results obtained with guinea-pigs are also found in human beings, then apart from other non-specific stress conditions, the significant rise in the histamine level of the blood may serve as an index for diagnosis of subclinical ascorbic acid deficiency.

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