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Role of L-ascorbic acid on detoxification of histamine

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It has been reported by Longenecker *et al.*[1] and Conney and Burns[2] that administration of a number of drugs, quite unrelated in structure and pharmacological action, stimulated synthesis of L-ascorbic acid in rats. Results obtained in this laboratory indicate that the enhanced synthesis of L-ascorbic acid might be related to an induced formation of histamine in the system.[3] Experiments using phenobarbitone, chlorpromazine and meprobamate revealed that the stimulation of L-ascorbic acid synthesis was accompanied by a concomitant increase (about 4–6-times) in urinary excretion of histamine. This enhanced urinary excretion of histamine is probably due to an elevated histamine level in the system due to induced histamine formation[4] or to inhibition of *N*-methylation of histamine by the above mentioned drugs[5] or both. We considered that the production of excess L-ascorbic acid in

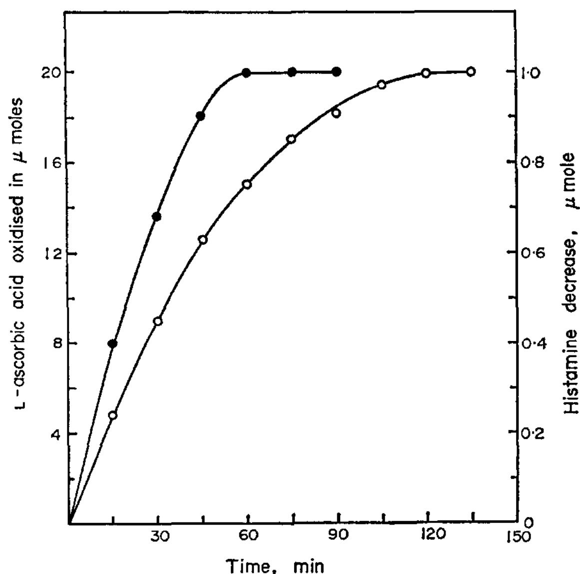

Fig. 1. Oxidation of L-ascorbic acid and biotransformation of histamine. The concentrations of L-ascorbic acid, Cu^{2+} and histamine and other conditions were same as Table 1. ●—●, oxidation of L-ascorbic acid; ○—○, decrease of histamine.

TABLE 1. EFFECT OF OXIDATION OF L-ASCORBIC ACID ON BIOTRANSFORMATION OF HISTAMINE

Additions*	Decrease in histamine (%)
None	Nil
L-ascorbic acid	1
Cu^{2+}	1
H_2O_2	1
$Cu^{2+} + H_2O_2$	1
Cu^{2+} + L-ascorbic acid	100
Dehydroascorbic a cid	Nil
Dehydroascorbic acid + $Cu^{2+} + H_2O_2$	1

* Concentrations used; histamine, 1 μmole; Cu^{2+} as $CuSO_4$, 0·1 μmole; H_2O_2, 40 μmoles; L-ascorbic acid and dehydroascorbic acid, 20 μmoles each. Final volume 2·5 ml, incubated in 0·04 M citrate–phosphate buffer at pH 5·5 for 2 hr at 37°.

response to an elevated histamine level might be a natural defence mechanism for detoxicating the excess histamine. To trace this, we examined the effect of ascorbic acid on histamine both *in vitro* and *in vivo*.

Urine was collected into 4 ml of 6N HCl and histamine was extracted and estimated fluorimetrically using *O*-phthalaldehyde by the method of Shore and Burkhalter.[6] In a recovery experiment adding 2–10 μg of histamine to 5 ml of rat or guinea pig urine or tissue homogenates, about 90 per cent of the added histamine was estimated by this method. Ascorbic acid was estimated titrimetrically using 2,6-dichlorophenolindophenol.[3]

The results indicate that L-ascorbic acid when added to a system containing histamine and a catalytic amount of Cu^{2+}, the histamine disappeared concurrent with the oxidation of L-ascorbic acid (Fig. 1). In the absence of Cu^{2+}, neither L-ascorbic acid or the products of its aerobic oxidation, dehydroascorbic acid and hydrogen peroxide,[7] were effective in reacting with histamine under experimental conditions (Table 1). It would appear that monodehydro ascorbic acid,[8] an intermediate in the oxidation of L-ascorbic acid into dehydroascorbic acid, might be the reactive compound.

TABLE 2. BIOTRANSFORMATION OF HISTAMINE INCUBATED WITH DIFFERENT RAT TISSUE HOMOGENATES IN THE PRESENCE OF L-ASCORBIC ACID

Tissue	Addition	Ascorbic acid oxidized (%)	Decrease in histamine (%)
Kidney	None		10
	L-ascorbic acid	70	73
Blood	None		10
	L-ascorbic acid	92	61
Liver	None		8
	L-ascorbic acid	72	51
Brain	None		12
	L-ascorbic acid	23	25

The incubation system contained 0·05 M sodium–phosphate buffer, pH 7·2, 0·25 μmoles of histamine, 5 μmoles of L-ascorbic acid when added and 2·5 ml of blood or a 20 per cent tissue homogenate in 0·05 M sodium–phosphate buffer, pH 7·2, in a final volume of 5 ml. After incubation for 30 min at 37°, the reaction was stopped by addition of 2·4 M $HClO_3$ for estimation of histamine. When L-ascorbic acid was estimated, 5% HPO_4 was used in place of $HClO_4$.

Thin layer chromatography of the reaction product on Silica gel G, using 95% ethanol–25% ammonium hydroxide (80:20) as the developing solvent, failed to detect the imidazole moiety by spraying with Pauly reagent.[9] Imanaga has shown that auto-oxidation of L-ascorbic acid in the presence of the imidazole moiety leads to rupture of the imidazole ring with evolution of ammonia.[10] However, the mechanism is not clear at present. This system is apparently different from the ascorbic acid mediated aromatic hydroxylating system of Udenfriend *et al.*[11] in that EDTA was inhibitory and Fe^{2+} was almost ineffective in catalyzing the chemical transformation of histamine.

Six male guinea pigs, approx. 200 g body wt, were injected subcutaneously with 10 μmoles of histamine, preincubated for 2 hr with 1 μmole of Cu^{2+} and 200 μmoles of L-ascorbic acid. This did not produce any syndrome of histamine shock, whereas omission of L-ascorbic acid resulted in the immediate death of all six control guiena pigs due to anaphylactic shock. However, L-ascorbic acid alone administered intraperitoneally at a dose as high as 1 g/kg body wt, could not prevent the death of the animals by subcutaneous injection of 5 μmoles histamine. This was apparently because the anaphylactic shock appeared within 5–7 min after the injection of histamine and in this short period the oxidation of injected ascorbic acid was not sufficient to detoxicate the histamine.

In the presence of blood and homogenates of liver, kidney and brain, L-ascorbic acid was readily oxidized (Table 2) and addition of Cu^{2+} was not necessary. Histamine added to such a system could only be partially recovered. The oxidation of L-ascorbic acid and the disappearance of histamine can be seen in Table 2.

Intraperitoneal injection of 1 and 5 mg of histamine to female rats of approx. 150 g body wt, resulted in urinary excretion of approximately 10 and 100 μg histamine/rat/day, respectively. This was reduced to about 2·5 μg (normal excretion level) and 15 μg/day respectively after oral administration of a single or divided dose of 100 mg L-ascorbic acid. When male guinea pigs, approx. 200 g body wt, were placed on a scorbutic diet for 10–12 days, the urinary excretion of histamine increased approx. 2-fold and the value returned to normal (about 2 μg/guinea pig/day) after administration of 5 mg L-ascorbic acid/guinea pig/day.

It has been reported by Schayer[12] that histamine formation in the system is markedly enhanced under different stress conditions, namely; infection, burns, cold and administration of a number of chemical compounds. Numerous reports have suggested that large doses of ascorbic acid are beneficial in various stress conditions.[13,14] In view of the results presented in this communication, it would appear that any beneficial effect of ascorbic acid is due to its detoxication of excess histamine produced in response to the stress.

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