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ASCORBIC ACID IS AN ABETTOR IN CALCIUM UROLITHIASIS : AN EXPERIMENTAL STUDY

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Abstract

Two sets of animal experiments using guinea pigs were planned to evaluate the effect of ascorbic acid supplementation on the lithogenic process. In the first set of experiments, 10, 40, and 60 mg doses of ascorbic acid/100g body weight/day were given for 105 days. Neither of the ascorbic acid doses given induced crystalluria, calcification or stone formation, thereby confirming our previous findings that ascorbic acid in the doses used by clinicians does not cause urolith formation. In the second set of experiments, ascorbic acid was supplemented in hypercalciuric (induced by calcium carbonate feeding) and hyperoxaluric (induced by sodium oxalate feeding) animals for 45 days. The results indicated that it exacerbated the calcification process in renal and bladder tissue.

KEY WORDS: Ascorbic acid, crystalluria, calcification, hypercalciuria, hyperoxaluria, urolith, sodium oxalate, calcium carbonate.

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Introduction

While the perils of ascorbic acid deficiency are well established, the dangers of its excessive intake especially in relation to oxaluria and urolithiasis is still a controversial issue. This needs to be carefully examined as there is an increasing tendency among the health conscious people of self medication with high ascorbic acid doses with a notion to tone up the body resistance system and by the clinicians to provide better protection against infection, cold, cancer, atherosclerosis, tuberculosis and metal poisoning (Pauling, 1970; Krupp et al . 1986). Also, ascorbic acid is considered as one of the safest and best chemical for acidifying the urine (Krupp et al. 1986).

A number of workers have reported hyperoxaluria on heavy ascorbic acid dosing (Chalmers et al. 1986; Pendse et al. 1985 and Schmidt et al. 1981). Although, to this date, there is no direct evidence of ascorbic acid dosing at any length of time leading to stone formation in human beings but Smith et al. (1978) suggested such a possibility in some renal stone patients. Briggs et al. (1973) felt that some individuals could be over sensitive to oxalate synthesis from ascorbic acid and might suffer from renal calcification/ stones on higher doses of ascorbic acid.

On the other hand, Hoffer (1971) questioned the validity of the above said belief on the basis of his experience stretching over 18 years in which he gave 3-30g ascorbic acid daily to over 1000 patients for prolonged periods. He did not come across a single case of stone formation or of any other serious toxicity. Our studies on rats also suggested the same view (Singh et al. 1988). Since the rat can synthesize ascorbic acid, we decided to extend our study to guinea pigs whose ascorbic acid metabolism is similar to man. In addition to this, we also examined its effect under the condition that when the diet contained a higher content of calcium, a condition frequently visible in the diet of western subjects and a higher content of oxalic acid, a condition occasionally seen in the Indian population (Singh et al. 1972, 1986 and 1990).

Material and Methods

Male guinea pigs (Cavia porcellus) of an inbred strain (Wt. 340-380 gm) were selected. They were given a standard basal diet, commencing two weeks prior to experimentation to mitigate the effect of their previous diet, if any, and during experimental period.

Experimental protocol. Animals were divided into 8 groups of 7 animals each. Group I - Basal diet; Group II - basal diet + 10 mg ascorbic acid /100 g body weight; Group III - basal diet + 40 mg ascorbic acid/100 g body weight; Group IV - basal diet + 60 mg ascorbic acid/100 g/body weight; Group V - basal diet + 2% Calcium carbonate; Group VI - basal diet + 2% calcium carbonate + 10 mg ascorbic acid/100 g/body weight; Group VII - basal diet + 3% sodium oxalate; Group VIII - basal diet + 3% sodium oxalate + 10 mg ascorbic acid/100 g/body weight. Sodium oxalate and calcium carbonate were given as a mixture with diet.

Food and water were given ad libitum. Food intake was noted daily and body weight was recorded twice a week. Physical and behavioural appearances were observed everyday. The ascorbic acid was dissolved in water and was orally given by introducing a 17 gauge endoesophagial tube into the stomach through the mouth and the solution was instilled using a syringe. The duration of study was 105 days in group 1-3 and 45 days in group 4-7.

Collection of urine samples. The initial 24 hr urine samples were collected before any supplementation. Post load samples were collected at interval of 15 days. 0.3 mmol EDTA (sodium salt) was added in collection bottles to prevent the conversion of ascorbic acid to oxalic acid (Chalmers et al. 1985). Urine samples were transferred to collection bottles. Trays were rinsed with double distilled water and contents were transferred to collection bottles. Final volume of all urine samples was raised to 25 ml. All the samples were analysed for creatinine, ascorbic acid, phosphorus, magnesium, uromucoid (Natelson, 1971), calcium (Gindler and King, 1972), uric acid (Caraway, 1955), citric acid (Rajagopal, 1984) and oxalic acid (Hodgkinson and Williams, 1972). The pH of fresh urine was measured by narrow range BDH pH strips.

Histological study. The histological sections of kidney and bladder were cut on the microtome and the sections were stained with Von Kossa solution.

Statistical evaluation was carried out by employing paired 't' tests.

Results

The results of the effect of supplementation of 10, 40, and 60 mg ascorbic acid/ 100 g body Wt./day are given in Tables 1-3. There was a significant drop in urinary pH after supplementation of ascorbic acid in all the experimental groups though at different periods of time. Higher doses of ascorbic acid (40 and 60 mg) induced increased oxalic acid excretion and decreased citric acid, calcium and magnesium excretion at different periods of time (Tables 2 and 3). Other parameters remained unaltered and so was the picture of crystalluria. The ascorbic acid excretion significantly increased in all the groups. The results of 2% calcium carbonate and 3% sodium oxalate with and without ascorbic acid supplementation are given in Tables 4-7. Their feeding increased calcium and oxalic acid excretion respectively. Ascorbic acid supplementation did not influence urine chemistry in either group but histological examination revealed that ascorbic acid intensified the renal and bladder tissue calcification in both the groups (Figs. 1-2). Urinary calcium/citrate and calcium/magnesium ratio remained unaltered on ascorbic acid

TABLE 1: Urine Chemistry (mg/24 hr) of Guinea Pigs Supplemented with 10 mg Ascorbic acid/100 gm Body Weight/day.

Days	pH	Creatinine	Calcium	Oxalic acid	Ascorbic acid	Uric acid	Uromucoid	Inorg. phos.	Magnesium	Citric acid
I	7.9±0.9	11.2±3.9	4.8±1.4	3.1±0.9	0.5±0.2	3.1±0.9	2.6±1.1	6.7±3.7	2.3±0.8	3.1±1.3
15	6.8±0.8a	11.0±4.2	4.9±1.4	3.2±0.9	1.4±0.2a	3.1±1.0	2.6±1.0	6.8±3.4	2.3±0.7	3.1±1.0
30	6.6±0.8	11.5±4.5	4.7±1.5	3.3±0.9	1.4±0.2b	3.2±1.2	2.5±1.0	6.7±2.3	2.4±0.9	3.2±1.1
45	6.6±1.0a	11.2±4.8	4.9±2.2	3.3±1.0	1.3±0.7b	3.0±0.9	2.5±0.9	6.6±3.2	2.2±0.7	3.0±1.0
60	6.9±0.9a	10.9±4.9	5.2±2.4	3.2±1.2	1.5±0.4b	3.2±1.2	2.5±1.0	6.7±2.2	2.4±0.9	3.2±0.9
75	6.5±0.8a	11.1±6.1	5.1±2.3	3.1±0.8	1.6±0.5b	2.9±1.1	2.7±0.8	6.6±2.5	2.3±0.9	2.9±1.0
90	6.4±0.9a	11.0±5.0	5.0±2.7	3.4±1.1	1.4±0.5b	2.9±1.6	2.7±0.8	6.7±2.8	2.2±0.6	2.8±1.1
105	6.5±1.0a	11.3±4.0	5.1±3.1	3.6±1.2	1.5±0.4b	3.0±1.4	2.8±1.8	6.8±2.2	2.2±0.7	2.8±1.1a

I - Initial; a - $p < 0.05$; b - $p < 0.02$

TABLE 2: Urine Chemistry (mg/24 hr) of Guinea Pigs Supplemented with 40 mg Ascorbic acid/100 gm Body Weight/day

Days	pH	Creatinine	Calcium	Oxalic acid	Ascorbic acid	Uric acid	Uromucoid	Inorg. phos.	Magnesium	Citric acid
I	8.0±0.8	10.3±2.5	4.9±1.5	3.0±1.1	0.4±0.02	2.8±1.1	2.5±1.0	6.0±2.3	2.4±0.9	2.2±0.7
15	5.8±0.8c	10.9±3.5	4.5±1.8	3.9±1.2	2.4±1.0c	3.0±1.0	2.4±1.1	6.4±2.3	2.2±0.8	1.7±0.8
30	6.0±0.9c	10.8±3.8	4.4±1.9	3.9±1.2	2.4±1.0c	2.7±1.1	2.9±1.4	6.3±3.1	2.0±0.8	1.6±0.6
45	5.8±0.7c	11.0±3.7a	4.7±2.1	4.0±1.1	2.5±1.0c	3.1±0.9	2.9±1.4	6.5±2.8	2.1±0.7	1.4±0.7a
60	6.2±0.8c	10.9±4.1	4.2±2.1a	4.3±1.2a	2.5±1.0c	3.0±0.9	2.7±1.0	6.8±3.2	1.8±0.7	1.3±0.5a
75	5.9±0.9c	10.9±4.3	4.1±2.0a	4.2±1.3b	2.6±1.0c	2.9±1.0	3.0±1.5a	6.7±2.9	1.2±1.0a	1.2±0.5b
90	5.3±0.4c	10.8±3.6	4.0±1.8a	4.8±1.9b	2.5±0.9c	3.1±1.4	3.0±1.5	6.8±3.2	1.1±0.4a	1.2±0.6b
105	5.4±0.3c	10.9±4.1	3.8±1.7b	4.8±1.9b	2.6±0.9c	3.8±4.4a	3.1±1.4	6.7±2.9	1.0±0.3c	1.0±0.4c

I - Initial; a - $p < 0.05$; b - $p < 0.02$; c - $p < 0.001$

TABLE 3: Urine Chemistry (mg/24 hr) of Guinea Pigs Supplemented with 60 mg Ascorbic acid/100 gm Body Weight/day

Days	pH	Creatinine	Calcium	Oxalic acid	Ascorbic acid	Uric acid	Uromucoid	Inorg. phos.	Magnesium	Citric acid
I	7.9±0.3	10.3±3.2	5.0±0.3	2.9±0.9	0.47±0.24	3.1±0.9	2.6±1.6	6.8±2.2	2.5±0.8	2.0±0.8
15	5.5±0.4c	10.4±4.1	4.6±2.0	3.8±1.1	3.9±0.9c	3.2±1.1	2.9±1.6	6.8±2.9	2.2±0.9	0.8±0.4a
30	5.5±0.3c	10.7±4.3	4.4±2.0	4.3±1.7a	4.4±0.8c	3.4±1.2	3.0±1.8	6.9±3.1	1.9±0.7a	1.0±0.3a
45	5.0±0.5c	10.3±4.9	4.0±1.8	4.5±2.1a	4.3±1.0c	3.2±1.4	3.2±2.0	4.6±2.8	1.8±0.8a	0.8±0.5b
60	5.5 0.6c	10.9±4.2	3.5±1.9a	4.9±1.7a	4.4±1.1c	3.0±0.9	3.9±1.0a	7.1±3.2	1.6±0.7b	0.8±0.4a
75	5.5±0.7c	10.3±5.5	3.7±1.9	4.8±1.3a	4.5±0.9c	3.3±0.9	3.5±1.3a	7.0±3.4	1.4±0.8b	0.9±0.4c
90	5.5±0.5c	10.6±4.8	3.4±1.4a	4.9±2.0a	4.4±1.7c	3.2±1.2	3.4±1.4	7.2±3.3	1.4±0.6b	0.6±0.3b
105	5.5±0.3c	10.7±4.5a	3.4±1.4a	4.9±2.1a	4.7±1.0c	3.4±1.6	3.4±1.3	7.0±3.0	1.2±0.7b	0.7±0.2c

I - Initial; a - $p < 0.05$; b - $p < 0.02$; c - $p < 0.001$

TABLE 4: Urine Chemistry (mg/24 hr) of Guinea Pigs Fed Normal Diet + 2 % Calcium Carbonate

Days	pH	Creatinine	Calcium	Oxalic acid	Ascorbic acid	Uric acid	Uromucoid	Inorg. phos.	Magnesium	Citric acid
I	8.0±0.9	9.8±3.3	4.9±2.0	3.3±0.9	0.5±0.3	3.3±1.2	2.3±0.9	6.8±2.8	2.3±0.4	2.4±0.6
15	7.5±0.8	10.6±4.4	6.2±3.0	2.8±1.6	0.4±0.2	3.7±1.9	2.5±1.2	6.6±2.4	1.6±0.4	1.6±0.7
30	7.6±0.6	10.8±4.1	6.4±1.9	2.6±1.4	0.4±0.2	3.8±2.0	2.7±1.3	6.8±3.8	1.2±0.4	1.3±0.5
45	7.5±0.7	10.5±3.9	6.5±1.9	2.8±1.5	0.5±0.2	3.6±1.7	2.9±1.3	6.7±2.9	1.2±0.4	1.2±0.5

I - Initial

TABLE 5: Urine Chemistry (mg/24 hr) of Guinea Pigs Fed Normal Diet + 2% Calcium Carbonate + 10 mg Ascorbic acid/100 gm Body Weight/day

Days	pH	Creatinine	Calcium	Oxalic acid	Ascorbic acid	Uric acid	Uromucoid	Inorg. phos.	Magnesium	Citric acid
I	7.9±0.6	10.2±4.1	4.6±2.1	3.2±0.8	0.4±0.1	3.5±0.8	2.4±0.9	6.6±2.9	2.2±0.7	2.6±0.8
15	7.3±0.8a	9.8±4.2	6.3±3.1	2.9±1.2	0.8±0.4a	3.8±1.3	2.4±1.1	6.8±2.4	1.4±0.5	1.4±0.6
30	7.3±0.6a	10.6±4.4	6.5±3.2	3.0±1.0	1.0±0.5a	3.9±1.4	2.9±0.9	6.9±3.2	1.2±0.4	1.3±0.6
45	7.2±0.8a	10.4±4.3	6.5±3.2	3.0±1.1	1.1±0.5a	3.9±1.4	3.1±0.9	6.7±2.8	1.1±0.3	1.0±0.5

I - Initial; a - $p < 0.05$

TABLE 6: Urine Chemistry (mg/24 hr) of Guinea pigs Fed Normal Diet + 3 % Sodium Oxalate

Days	pH	Creatinine	Calcium	Oxalic acid	Ascorbic acid	Uric acid	Uromucoid	Inorg. phos.	Magnesium	Citric acid
I	8.0±0.9	10.9±3.2	5.1±2.1	3.2±1.8	0.5±0.2	3.4±1.0	2.6±1.0	6.6±3.0	2.4±1.4	2.4±1.1
15	7.6±0.8	9.6±3.8	3.3±0.9	4.4±2.1	0.4±0.2	4.1±2.0	3.2±1.1	7.2±3.1	1.5±0.4	1.3±0.5
30	7.7±0.9	9.3±3.2	3.1±1.2	4.7±2.2	0.4±0.2	4.2±1.8	3.5±1.4	7.4±3.6	1.6±0.5	1.4±0.6
45	7.6±0.2	10.6±2.7	3.4±1.4	4.6±2.0	0.4±0.1	4.2±2.0	3.8±1.7	7.2±3.8	1.7±0.2	1.3±0.5

I - Initial

TABLE 7: Urine Chemistry (mg/24 hr) of Guinea Pigs Fed Normal Diet + 3 % Sodium Oxalate Along with 10 Ascorbic acid/100 gm Body Weight/day.

Days	pH	Creatinine	Calcium	Oxalic acid	Ascorbic acid	Uric acid	Uromucoid	Inorg. phos.	Magnesium	Citric acid
I	8.0±0.1	10.0±3.8	4.8±2.2	3.8±1.8	0.5±0.1	3.8±1.8	2.4±0.9	7.1±3.5	2.4±1.3	2.5±0.1
15	7.2±0.5	10.5±4.1	3.0±1.1	4.9±1.3	1.0±0.4a	4.4±1.3	4.1±2.0	7.7±3.2	1.3±0.4	1.2±0.3
30	7.3±0.6a	10.4±4.0	3.0±0.9	4.9±2.0	0.9±0.4a	4.2±1.4	4.3±1.8	7.9±3.0	1.3±0.3	1.2±0.4
45	7.2±0.3a	10.8±2.9	3.2±1.0	4.9±1.8	0.9±0.5a	4.3±1.9	4.0±2.0	7.6±2.9	1.4±0.5	1.2±0.7

I - Initial; a - $p < 0.05$

supplementation in sodium oxalate and calcium carbonate fed groups.

Discussion

The majority of urinary stones contained calcium oxalate (Smith, 1989) including those analysed by us (Hada et al. 1989; Pendse and Singh 1986; Pendse et al. 1987, 1984 and Singh et al. 1978). It is also unquestionably determined that oxalic acid is the most cogent risk factor in stone formation (Borsatti, 1991; Menon and Mahle, 1982).

In human beings, ascorbate is the major source of oxalate (Hodgkinson, 1977). It is believed that the harmful effect of ascorbic acid in the human body lies in its conversion to oxalic acid generating a fear that higher intake may lead to enhanced oxalate production and consequently synthesis of stones in urinary tract (Smith et al. (1978). Nevertheless, the issue remained unresolved because of the lack of any direct evidence of its involvement in the formation of stone in man or animal (Hoffer, 1971 and Singh et al. 1988).

In our extended experiment reported herein, 10, 40, and 60 mg doses of ascorbic acid per kg body weight were given to guinea pigs. These doses theoretically correspond to 6, 24 and 36 g dose for a 60 Kg person and are in conformity with the doses advocated and given by many clinicians (Pauling, 1970; Hoffer, 1971). The experiment was conducted for 15 weeks. Taking the average life of guinea pigs as 3 years and that of man as 60 years, this period again theoretically corresponds to 7 years of human life. The normal ascorbic acid requirement of guinea pig is 0.5 mg/100 g body wt./day (Collins

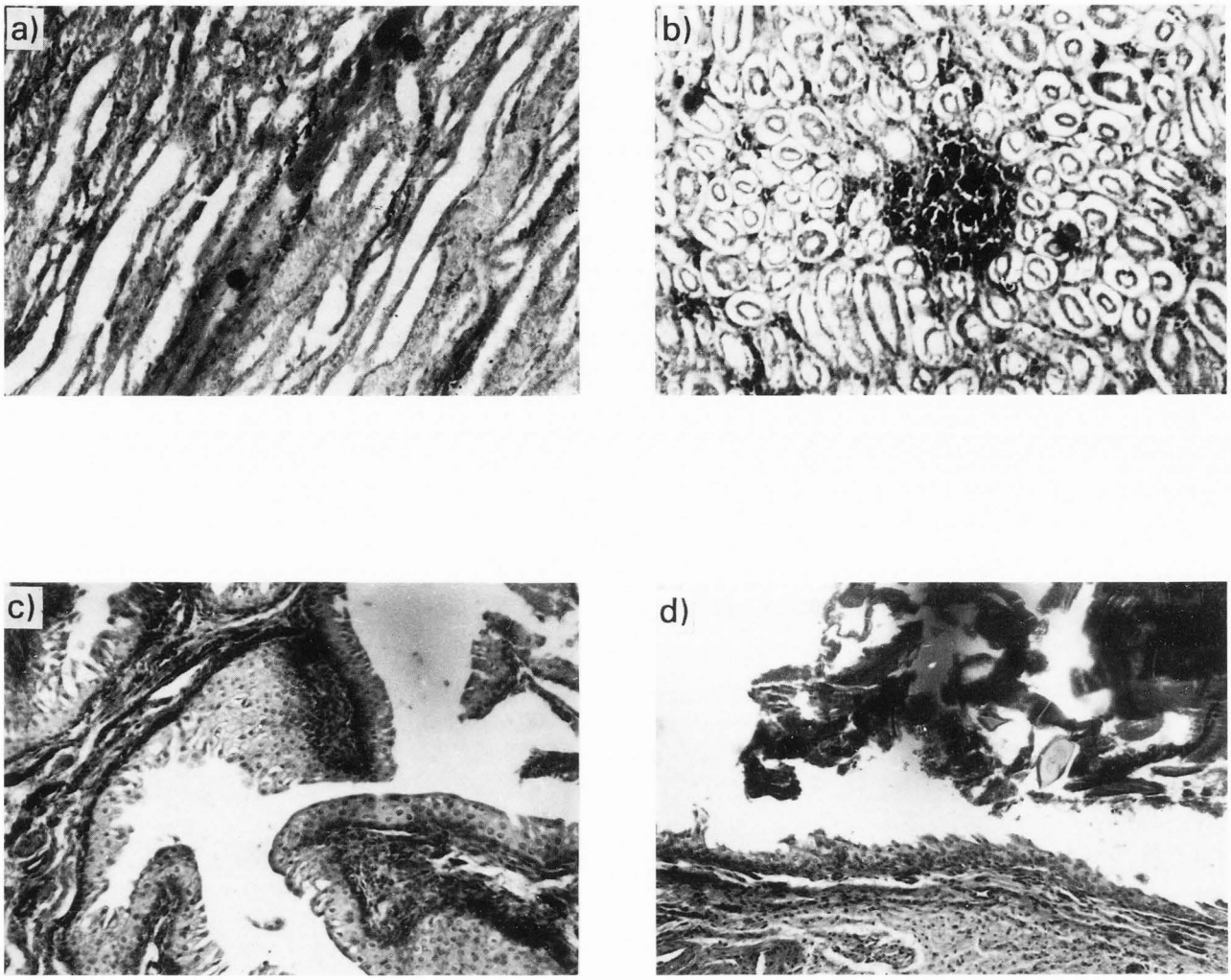

Figure 1(a). Calcium deposits in lumen of kidney tubule of guinea pigs fed sodium oxalate. (b) Intraluminal calcium deposits in kidney tubular and interstitial tissue of guinea pigs fed sodium oxalate + ascorbic acid. (c) No effect of feeding sodium oxalate on bladder tissue. (d) Dense calcium deposits in cavity of bladder tissue of guinea pigs fed sodium oxalate + ascorbic acid.

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and Elvehjen, 1958. Thus the selected doses of ascorbic acid were 20, 80 and 120 times to the normal requirement. This period and the doses were therefore, considered sufficient to judge the effect of chronic toxicity of this vitamin.

Ascorbic acid showed its potential value in acidification of urine. Forty and 60 mg doses did affect the urinary profile. The possible explainations for these changes have already been given earlier (Singh et al. 1988 and 1987). The gross and histological examination did not indicate any crystal deposition, stone formation or calcification in kidney, ureter or bladder.

All the findings taken together reconfirm our conclusions derived from rat experiments that ascorbic acid alone does not cause stone formation though it can increase oxaluria in higher doses. Since crystallization and aggregation process is dependent on net state of urinary chemical milieu; and that trapping of these crystals or aggregated crystals on a specific site of urinary tract depends on assembly of multiple factors (Pak, 1987), it shall therefore be unreasonable to assume that hyperoxaluria alone can lead to stone formation.

However, no one can deny the fact that hyperoxaluria makes urine propitious to stone formation. It, therefore, would be logical to presume that in a stone former with preexisting stone risk, it will add to supersaturation and, therefore, can initiate stone formation or can cause renal calcification. Thus, ascorbic acid under such conditions may cause adverse effects. This would possibly explain the reason why in some patients metabolically inactive stone disease became active and also they developed new stones after regular consumption of ascorbic acid (Smith, et al. 1978).

To verify the above hypothesis, we planned two experiments. In one set, a high calcium diet

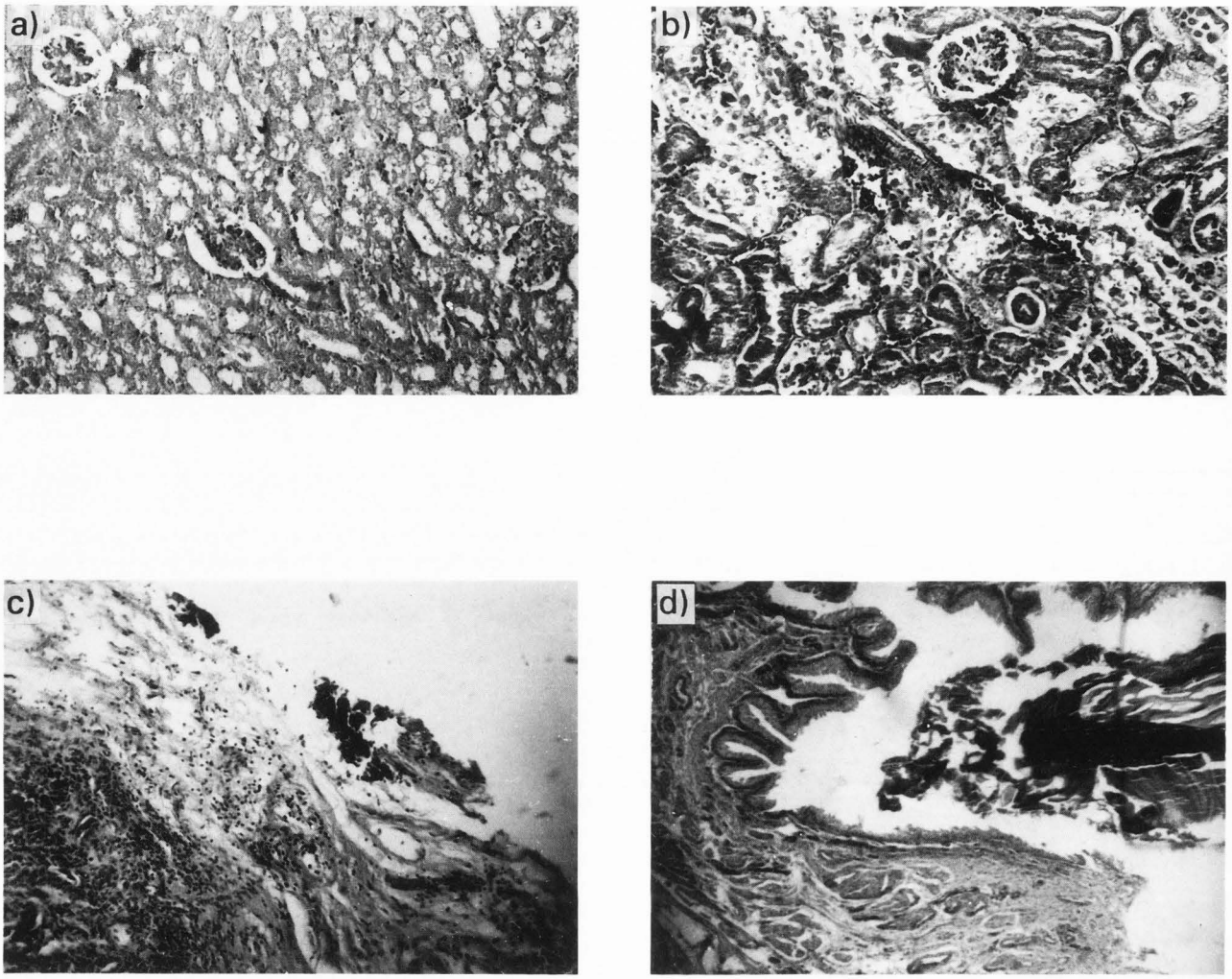

Figure 2 (a). Intraglomerular calcium deposits in guinea pigs fed calcium carbonate. (b) Intraglomerular and interstitial tissue calcium deposits in guinea pigs fed calcium carbonate + ascorbic acid. (c) Ulceration of mucosa with calcium deposit in the bladder of guinea pigs fed calcium carbonate. (d) Dense calcium deposits in the cavity of bladder of guinea pigs fed calcium carbonate + ascorbic acid.

was given to animals. Half of these animals were also given 10 mg ascorbic acid/100 g body wt./ day. This experiment was planned keeping in view that in an affluent population, especially in industrialized countries, calcium intake is generally high. Calcium supplementation alone decreased the urinary oxalic acid significantly which should be due to decreased availability of oxalic acid for absorption in the intestine (Williams and Wandzilak, 1989). In the ascorbic acid supplemented group, however, oxalic acid excretion remained unchanged suggesting the possibility that decrease in oxalate absorption could have been compensated by conversion of part of ascorbic acid to oxalic acid. The histological findings revealed that ascorbic acid supplementation did not induce stone formation but intensified the renal calcification.

During some periods of the year, oxalate intake is fairly high in this region and some other parts of India (Singh et al. 1990, 1986 and 1972; Hodgkinson, 1977) and is considered to be etiologic factor (Pendse et al. 1987 and 1984; Singh et al. 1992 and 1978). The ascorbic acid supplementation did not alter the urine chemistry in this set up also except that ascorbic acid excretion increased and pH decreased. However, histological examination once again showed the increased renal and bladder tissue calcification.

The data of these two experiments taken together do suggest that ascorbic acid ingested in large doses increases the risk of renal calcification and chances of stone formation in preexisting hypercalciuric and hyperoxaluric conditions.

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Discussion with Reviewers

A.Hesse: Are the 3 % sodium oxalate given as a mixture with the food or dissolved in the drinking water (ad libitum)?

Authors: It was given with diet.

A.Hesse: Is it possible to establish a calcium and oxalate balance according to the intake of calcium and oxalate with the food?

Authors: No. This study was not designed for this purpose.

A.Hesse: What is the 24 hr urine volume of the animals like?

Authors: Twenty four hr urinary output was not

recorded because all the samples were collected in a bottle and material left behind was rinsed with double distilled water. Samples were finally made to 25 ml.

A.Hesse: Is it possible to calculate stone risk factor (for example risk factor according to Robertson, the EQUIL-program by Finlayson or the calcium/citrate quotient)?

Authors: Urinary calcium/citrate and calcium/magnesium remained unaltered on ascorbic acid supplementation in sodium oxalate and calcium carbonate fed groups. Hence these data are not included in the tables.

S.R.Khan: Did you look for crystalluria?

Authors: Yes. Ascorbic acid in neither of the doses affected urinary crystalluria.

S.R.Khan: Whether the calcification is intraluminal or intracellular and whether it was due to calcium phosphate or calcium oxalate?

Authors: It is intraluminal. Since we have not examined the crystals under polarised microscope, we cannot authentically comment on it.