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The Influence of Ascorbic Acid on Blood Pressure (Part III)

THE EFFECT OF ASCORBIC ACID ON THE BLOOD PRESSURES OF THE
RATS FED WITH HIGH SODIUM CHLORIDE DIETS

BY

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In the preceding paper the author reported that, in the rice-producing districts of the Kitakami River basin, the concentration of ascorbic acid in the sera of the farmers showed a considerably low value in winter season, and this might be considered to be one of the factors which caused their hypertension¹⁾.

The effect of a large amount of dosage of ascorbic acid on hypertension has already been reported by some investigators but the way in which it works on the blood pressure has not yet been made clear. Ascorbic acid has an ability to activate the various enzymes in the liver²⁾, and from this it might be surmised that ascorbic acid serves to lower the blood pressure as it strengthens the activity of enzymes, such as amine-oxidase, which has a part in detoxicating the pressor amines produced in the kidneys, intestine and so forth.

Myasnikov²⁻⁴⁾ reported that ascorbic acid reduced blood cholesterol and inhibited the growth of fatty deposits in the aorta. This also might be regarded as a way in which ascorbic acid prevents arteriosclerosis and high blood pressure.

The author made an experiment to clarify whether or not a large amount of ascorbic acid dosage would serve to prevent the rise of the blood pressure of albino rats which were fed with high salt diets, and if it could, in what ways would ascorbic acid influence blood pressure, sodium and cholesterol in serum, enzymes in liver, and vitamins in liver and kidney.

EXPERIMENTAL METHOD

Twenty female albino rats of Wister strain, two-month-old, were used for the experiment and were housed in individual wide-screen-cages.

The rats were divided into four groups and two groups of them (Groups I-II) were fed with the diet containing 1% Sodium Chloride, while the

other two groups (Groups III-IV) were fed with the diet of 2.5% Sodium Chloride. Besides, 2 mg% of ascorbic acid crystal, dissolved in 5 ml water every other day, were given with the diet to each animal in groups II and IV. The basal diet of 100 g consisted of casein 8 g, Calcium Carbonate 1 g, Sodium Chloride 1 g or 2.5 g, thiamine 0.1 mg, riboflavin 0.1 mg, and the rest was polished rice flour. Three drops of liver oil *per capita* were also given once a week. The diet, which was prepared with 180 ml boiling water per 100 g of the diet, was given *ad libitum* and drinking water was not allowed.

The 24 hours' output of urine of each animal was collected in a flask containing hydrochloride acid after 14 weeks' feeding and the amounts of 17-ketosteroids in the urine was estimated by the method of Drektor et al.⁵⁾. In the twentieth week the systolic blood pressure of the rats were measured by the method of Williams et al.⁶⁾, and then the animals were decapitated. Immediately after the decapitation, the livers were excised, weighed and cooled with ice. One gram of the liver was homogenized in a Potter-Elvehjem's homogenizer with 9 ml of M/15 phosphate buffer (pH 7.2) to serve for the detection of the enzyme activities. The liver succino oxidase and mono-amine oxidase were determined by the method of Schneider and Potter⁷⁾ and Blaschko⁸⁾ respectively. The ascorbic acid and riboflavin contents in the livers were measured by the method of Fujita⁹⁾ and Yagi¹⁰⁾ respectively.

The livers and kidneys were fixed with 10% formaline solution and examined histologically.

The blood expelled from the cervical vessels at the decapitation was collected and the serum cholesterol was estimated by the method of Zuckerman et al.¹¹⁾ and the serum sodium by Tasaka et al.¹²⁾.

RESULTS

Growth of the Animals The weight of all animals

TABLE I BODY WEIGHT, LIVER WEIGHT AND BLOOD PRESSURE OF RATS

Group	Condition	No. of Rat	Initial body weight g	Final body weight g	Liver weight g	Ratio of liver wt. to body wt. %	Blood pressure mmHg
I	1 % Sodium Chloride diet	1	108	192	4.60	2.39	100
		2	106	171	3.90	2.28	106
		10	74.5	173	3.70	2.14	100
		11	72.5	166	3.70	2.23	96
		12	83	190	4.71	2.48	100
		Ave.	88.8	178.4	4.12	2.30	100.4
II	1 % Sodium Chloride diet plus As. A.	3	112	205	5.35	2.61	100
		4	97	198	4.75	2.40	96
		15	85	200	4.95	2.48	100
		16	82	181	4.19	2.32	100
		17	89	207	5.11	2.47	104
		Ave.	93.0	198.2	4.87	2.46	100.0
III	2.5% Sodium Chloride diet	5	117	184	5.00	2.72	110
		6	91	182	5.75	3.16	116
		7	70	195	5.32	2.73	106
		13	80	183	4.91	2.69	110
		14	72	160	4.65	2.90	106
		Ave.	86.0	180.8	5.13	2.84	109.6
IV	2.5% Sodium Chloride diet plus As. A.	8	82	217	5.37	2.48	106
		9	77	206	4.75	2.30	106
		18	102	209	5.38	2.57	100
		19	124	202	5.95	2.95	100
		20	86	199	—	—	—
		Ave.	94.2	206.6	5.36	2.58	103.0

kept increasing for the first 13 weeks of feeding, but after that the groups without ascorbic acid did not show any further increase in body weight. On the other hand, the groups supplemented with ascorbic acid showed a continual increase in weight, if only slightly. The weight of the liver was greater in the groups of high diet than those of low salt diet. The weight rate of the liver to the body was larger in the groups of high salt diet than those of low salt diet, and hypertrophy of the livers was observed in the former groups. The results are given in Table I.

Blood Pressure The rats with high salt diets showed slightly higher blood pressures than those with low salt diets. In the latter groups no effect of ascorbic acid added to the diet was observed on the lowering of blood pressure, while in the groups with high salt diets ascorbic acid was more or less effective. This difference, however, was not significant. The results are shown in Table I.

17-Ketosteroids in Urine The amounts of 17-ketosteroids in the urine averaged 28.34 γ /day (23.8–32.5 γ) in Group I, 26.02 γ (19.7–34.3 γ) in Group II, 30.51 γ (27.9–37.3 γ) in Group III, and 27.66 γ (25.4–27.8 γ) in Group IV. In all groups except Group IV, the value varied to a considerable extent.

Cholesterol and Sodium in Serum The concentration of cholesterol and sodium in the serum was generally higher in the groups of high salt diet. Of the two groups which were given the salt diet

of the same concentration, the group with ascorbic acid showed rather lower concentration of blood cholesterol. These differences, however, were of no significance. The estimation of the concentrations of serum sodium could not be made on all the rats, because very little blood was collected from some of the rats. The concentrations of cholesterol and sodium in the serum are given in Table II.

TABLE II SERUM CHOLESTEROL CONCENTRATION AND SERUM SODIUM CONCENTRATIONS OF RATS

Group	Condition	No. of Rat	Serum Cholesterol mg/dl	Serum Sodium mEq/l
I	1 % Sodium Chloride diet	1	56	140
		2	60	—
		10	53	140
		11	40	137.5
		12	50	141
		Average	51.8	139.6
II	1 % Sodium Chloride diet plus As. A.	3	54	135.5
		4	41	—
		15	60	142
		16	—	135
		17	47	140
		Average	50.5	138.1
III	2.5% Sodium Chloride diet	5	66	138.5
		6	70	139
		7	65	140.5
		13	60	135
		14	63	130.5
		Average	64.8	138.7
IV	2.5% Sodium Chloride diet plus As. A.	8	60	135
		9	60	138
		18	46	137
		19	54	137
		Average	55.0	136.8

TABLE III LEVELS OF ASCORBIC ACID AND RIBOFLAVIN, AND ACTIVITIES OF SUCCINO OXIDASE AND MONO-AMINE OXIDASE IN LIVER OF RATS

Group	Condition	No. of Rat	Liver ascorbic acid mg/g	Liver riboflavin γ/g	Liver succino oxidase μl	Liver mono-amine oxidase μl
I	1 % Sodium Chloride diet	1	0.21	—	22.7	17.1
		2	0.25	—	22.0	15.2
		10	0.25	17.0	28.9	17.3
		11	0.24	27.0	20.6	18.0
		12	0.24	25.0	21.5	20.1
		Ave.	0.238	23.0	24.1	17.5
II	1 % Sodium Chloride diet plus As. A.	3	0.28	—	22.2	18.3
		4	0.24	—	19.6	16.5
		15	0.28	28.0	26.3	14.6
		16	0.27	24.0	30.2	12.2
		17	0.27	21.0	27.5	18.0
		Ave.	0.268	25.3	25.2	17.9
III	2.5% Sodium Chloride diet	5	0.26	—	19.6	14.5
		6	0.18	—	26.3	17.3
		7	0.24	—	27.5	13.6
		13	0.20	21.0	27.0	18.5
		14	0.29	24.0	22.0	16.0
		Ave.	0.234	25.2	22.5	16.0
IV	2.5% Sodium Chloride diet plus As. A.	8	0.24	—	25.5	17.3
		9	0.26	—	27.5	17.6
		18	0.25	25.0	20.6	14.0
		19	0.24	21.0	19.6	18.0
		Ave.	0.247	23.0	23.3	16.7

The values are represented in O₂μl consumed per 10 min. per 20 mg of liver.

Ascorbic Acid and Riboflavin in Liver In the animals fed with 1 per cent salt diets supplemented with ascorbic acid (Group II), a slightly high level of ascorbic acid in the liver was found. However, no difference was observed in the amounts of ascorbic acid and riboflavin in the livers of all the groups (Table III).

Enzyme Activity in the Liver As is shown in Table 3, no distinct differences in the activities of the liver succino oxidase and mono-amine oxidase were seen among the four groups.

Patho-Histological Changes The author observed the hyperemia of the liver in all groups, the infiltration of the lymphoid cells in some of the animals on 2.5% salt diet, especially in Group III, and the greater changes of the capillary loops in the groups of 2.5% salt diet. These histological figures of the kidneys are shown in Table IV and Fig. 1.

DISCUSSION

It has been supposed that, rats, mice, rabbits, and so on could synthesize ascorbic acid in themselves and grow normally without supplies of ascorbic acid from their foods. It has been, however, reported that the contents of the adrenal ascorbic acid were considerably lower in the albino rats fed with ascorbic acid deficient synthetic diets than in

TABLE IV HISTOLOGICAL FINDINGS OF KIDNEY

Group	Condition	No. of Rat	Capillary loop	Lobulation of glomerulus*	Adhesions between tuft and capsule	Swelling of glomerulus	Thickening of blood vessels	Infiltration of lymphoid cell	Hyperemia	Proliferation of connective tissue	Cloudy swelling of lobular epithelia
I	1 % Sodium Chloride diet	1	—	—	—	—	—	—	+	—	—
		2	—	—	—	—	—	—	+	—	—
		10	—	—	—	—	—	—	+	—	—
		11	+	—	+	—	—	—	+	—	±
		12	—	—	—	—	—	—	+	—	±
II	1 % Sodium Chloride diet plus As. A.	3	—	—	—	±	—	—	+	—	—
		4	—	—	—	±	—	—	+	—	—
		15	±	±	±	—	—	—	+	—	—
		16	+	+	+	+	—	—	+	—	±
		17	—	—	—	±	—	—	+	—	—
III	2.5% Sodium Chloride diet	5	±	—	—	—	—	—	+	—	+
		6	±	±	±	±	±	+	+	—	—
		7	±	+	+	±	±	+	+	—	—
		13	±	±	±	±	—	+	+	—	—
		14	+	±	±	—	—	—	+	—	—
IV	2.5% Sodium Chloride diet plus As. A.	8	±	±	±	+	+	+	+	+	—
		9	±	±	±	—	—	—	+	—	—
		19	+	±	±	—	—	—	+	—	+

* It means that capillary loops adhere one another and form a few lamps.

the ones on stock diets of laboratory checkers¹³⁾, and that their growth was promoted by the administration of a large amount of ascorbic acid¹⁴⁾. A decrease of the adrenal ascorbic acid contents was reported in the rats which were exposed to the

Group I, No. 1

Group II, No. 16

Group III, No. 7
(Stained by Hematoxylin and Eosin)

Group IV, No. 8

Fig. 1. Histological Findings of Kidney

cold¹⁵⁾ and a decrease of the blood ascorbic acid contents was observed in the rabbits that were fed with an excessive administration of salt¹⁶⁾. It is naturally understood that the sensitivity of anti-scorbutic animals such as rats and rabbits to various stresses are different from that of monkey, guinea-pigs and human beings.

In the present study, the influence of ascorbic acid administration on the blood pressure of the rats fed with a high salt diet was examined.

The weight of the liver of the animals on the diet containing the same salt concentration, was greater in those with ascorbic acid than those without the vitamin. This was natural because the body weight of the animals supplemented with ascorbic acid were comparatively greater, but the weight ratio of liver to body was generally greater in the high salt diet groups, especially in the groups without ascorbic acid. Kimura et al.¹⁷⁾ observed that the swelling of the kidneys in the rats was

caused by the excessive intake of salt, and that the swelling could be prevented to some extent by the administration of ascorbic acid, riboflavin, or choline.

Takahashi¹⁸⁾ observed the liver swelling of the rats caused by riboflavin deficiency. One of the characteristics of ascorbic acid or riboflavin which prevents the hypertension induced with high salt diet, is probably that it serves to prevent the liver swelling. As is shown in Table II, ascorbic acid had no influence on the blood pressure of the rats on 1% salt diet, whereas a slight influence of ascorbic acid was observed in the groups of the high salt diet. An adequate amount of sodium¹⁹⁾²⁰⁾ and unilateral renalectomy²¹⁾ are necessary for elevating of blood pressure, and hormonal factors are said to have a close relationship to this. Heroux and others studied the effect of ascorbic acid on the experimental hypertension of rats, and reported that ascorbic acid prevented the hypertensive action of desoxycorticosterone acetate²²⁾²³⁾ and the injection of

ascorbic acid lowered the elevated blood pressure of rats which were exposed to the cold, or were given excessive salt in drinking water, or by the renal stricture²⁴⁾. While they studied the effect of ascorbic acid on the hypertension which had already been produced artificially, the author studied it by administering a sufficient amount of ascorbic acid with the diet from the outset. As reported previously, the concentration of the serum ascorbic acid was considerably low in the farmers of the area, where the frequency of hypertension is high. The deficiency of the vitamin for a long period is conjectured to be one of the factors which induce the hypertension and therefore, ascorbic acid should be regarded as a preventive factor for hypertension or arteriosclerosis rather than a therapy. As the hypertensive action of ascorbic acid can be considered to function through the adrenal-system, especially through its cortex²²⁾, the adrenal function was examined by the determination of 17-ketosteroids excreted in the urine, although the true function cannot be judged directly by the amount of urinary 17-ketosteroids.

Female rats were used for the experimental animal, because the urinary excretion of 17-ketosteroids in females is said to depend almost entirely on the activity of the adrenal cortex. The amounts of 17-ketosteroids in the urine varied day after day, and therefore, no difference between the experimental groups and the control groups was found. But the amount was a little smaller in the groups with ascorbic acid added to the diet.

Kimura et al.¹⁷⁾ observed that the urinary 17-ketosteroids of the rats on 3.5% salt diet, was much more than that of the normal diet group, and that simultaneous dosage of ascorbic acid was useful in decreasing the amount of urinary of 17-ketosteroids of rats on 3.5% salt diet.

Different observations have been reported concerning the influence of ascorbic acid on adrenal function²⁵⁻²⁸⁾ and they are suggesting that ascorbic acid has no influence on the urinary 17-ketosteroids in men, that the urinary excretion of 17-ketosteroids are diminished in ascorbic acid deficient guinea-pigs and that on the other hand the amounts are increased in scorbutic guinea-pigs. Bacchus and co-workers²⁵⁾³⁰⁾ reported the ascorbic acid prevented an increased excretion of 17-ketosteroids following cortisone treatment. Hirose³¹⁾ reported that the increased excretion of urinary 17-ketosteroids in rats

caused by the cholesterol administration could be lowered by the injection of ascorbic acid. These data and the results of the present experiment can be explained by Bacchus' report²⁵⁾, demonstrating that ascorbic acid prevents a decomposition of adreno-cortical hormones to 17-ketosteroids and reduces the urinary excretion of these hormones.

The slightly increased levels of the serum cholesterol in the animals with the 2.5% salt diet were observed and the slight effect of ascorbic acid on the serum cholesterol was noted.

Few studies have been reported on the changes of the blood cholesterol contents caused by an excessive intake of salt. Koyanagi et al. have reported the increase of the serum cholesterol contents of rats fed with a high salt diet³²⁾. As hypercholesterolemia is often observed in nephrose syndrome, the increase of the cholesterol contents in the present study may possibly be due to the renal lesions caused by the excessive salt intake, and therefore, the cause of the increase must be judged in reference to its patho-histological findings.

Kimura and coworkers³³⁾ estimated the serum cholesterol and ascorbic acid of the farmers in the rice-producing districts of Iwate-Ken, and found that there was no significant correlation between the two. Gillum and others³⁴⁾, on the other hand, reported a slightly positive correlation between the two components in older women. Myasnikov³⁾ observed that the continued use of ascorbic acid by patients with hypertony and atherosclerosis lowered the cholesterolemia and the vitamin inhibited experimental cholesterol atherosclerosis³⁾⁴⁾ and epinephrine-thyroxine sclerosis³⁵⁾ in rabbits. Moreover, there was a report, suggesting that ascorbic acid prevented the rising of serum cholesterol in rats on a high salt diet¹⁷⁾. On the contrary, Booker et al. reported that the dosage of the vitamin increased the serum cholesterol in rats and dogs³⁷⁾. Judging from these findings, the author has assumed that ascorbic acid has a normalizing action on serum cholesterol.

No significant difference was found among the serum sodium contents of the four groups.

It has been reported that ascorbic acid activates succino oxidase³⁷⁾, and the activity of cardiac succino oxidase decreases moderately in the experimental hypertensive rats of the Goldblatt type, while that in the liver does not change³⁸⁾. In the present experiment, however, no significant differ-

ence was recognized in liver succino oxidase.

SUMMARY

The influence of ascorbic acid on the metabolic phases was investigated in female rats which were fed with the diets containing 1.0 or 2.5 per cent Sodium Chloride and the following results were obtained.

1) A slight increase of blood pressure was observed in the animals on 2.5 per cent salt diets, compared with those on 1 per cent salt diets or 2.5 per cent salt diets supplemented with ascorbic acid.

2) Ascorbic acid which was previously added to the diet slightly decreased the urinary excretion of 17-ketosteroids and inhibited the rising of serum cholesterol in the animals on 2.5 per cent salt diets.

3) No significant difference were found in the serum sodium concentrations, the liver riboflavin and ascorbic acid contents.

4) No significant histological differences were seen in the kidneys of the animals fed with 1 per cent salt diets, either with or without the supplement of ascorbic acid. No differences, in the groups of 2.5 per cent salt diet, were of significance except the morphological changes in the capillary loops.

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