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

RELATIONS OF ASCORBIC ACID CONCENTRATION TO VITAMIN CONCENTRATIONS

AND MINOR COMPONENTS IN HUMAN BLOOD.

By

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1. RELATION OF ASCORBIC ACID CONCENTRATION TO RIBOFLAVIN, CHOLINE AND VITAMIN A CONCENTRATIONS IN HUMAN BLOOD.

In a previous paper (1), the author has reported that, determining the serum ascorbic acid concentrations of the farmers in the rice-producing districts of Iwate-Ken, there was a decrease in the ascorbic acid levels with age and a negative correlation between the vitamin level and blood pressure, and lower vitamin level was observed in the winter season.

Because it is known that some vitamins function in relationships to each other, it is important whether their mutual balances are maintained or not.

In the present study, the analyses of some vitamins which may influence blood pressure were carried out, and the relationships of ascorbic acid to the vitamins were examined.

EXPERIMENTAL METHODS

Chemical analyses of serum and blood vitamin levels were made on the inhabitants of the same village of the rice-producing districts as in the previous study.

Blood samples were secured at least two hours after meals and each subject was questioned to make sure no foods containing a large amount of ascorbic acid were taken in the meal prior to venipuncture.

Serum Ascorbic Acid Concentration

Immediately after the blood was collected from the cubital vein, the serum was separated by centrifugation, and ascorbic acid level was measured by the modified method (1) of Fujita's (2).

Serum Riboflavin Concentration

Blood collected from the cubital vein was kept in a dark-box, and the analysis of serum riboflavin was made by the method of Matsuura (3).

Blood Riboflavin Concentration

One ml of blood was taken into a 1.0 ml syringe moistened with sodium citrate solution. The syringe was emptied into a spitz tube and 2.0 ml of distilled water was added. The spitz tube was kept in a dark-box and the procedure was performed in the laboratory on the next day. The spitz tube was incubated at 80°C in a water bath for fifteen minutes, being stirred with a glass rod. After cooling it to room temperature, the mixture was separated by centrifugation, and each 1.0 ml of the supernatants was transferred into three glass-stoppered tubes made of nonfluorescent glass. The following procedure was made by the method of Yagi using microfluorometer (4).

Serum Choline Concentration

The sera used for the choline analysis was collected at the same time as those used for the ascorbic acid. Two ml of the serum was pipetted into a 100 ml beaker and 1.0 ml of choline solution (2.5 mg of choline chloride in water) was added.

After alkaline hydrolysis with barium hydroxide, the sample was neutralized to the phenolphthalein end point with 10 per cent acetic acid. Ten ml of 2 per cent solution of methanolic ammonium reineckate was then added to precipitate choline. The mixture was allowed to stand for one night in the cold to complete crystallization and then reineckate crystals were filtered out on a glass filter which was dried and washed with ethanol. The crystals were then dissolved in acetate. The colour intensity of the acetone solution was measured in a colorimeter at a wave length of 530 m μ . These procedures were made according to Jacobi's colorimetric reineckate method (5).

Serum Vitamin A Concentration

The level was measured by Sobel (6) using glycerol-dichlorohydrine.

EXPERIMENTAL RESULTS

1) *Ascorbic Acid and Riboflavin in Serum* On the ascorbic acid and riboflavin levels in serum, 15 males and 15 females were examined in August of 1955.

The data are given in Fig. 1. The correlation coefficient was only +0.285 and the correlation was not significant.

Fig. 1. Serum Ascorbic Acid and Serum Riboflavin Concentrations

TABLE I SERUM ASCORBIC ACID AND BLOOD RIBOFLAVIN CONCENTRATIONS

No.	Name	Sex	Ascorbic Acid mg%	Riboflavin γ%
1	S.Y.	m	0.96	7.6
2	C.F.	f	0.98	5.1
3	Y.F.	m	1.15	10.2
4	Y.O.	m	0.36	2.2
5	Y.S.	m	1.01	7.1
6	M.Y.	f	0.72	6.0
7	I.Y.	f	0.50	13.0
8	H.Y.	f	0.35	7.5
9	T.S.	m	0.30	5.1
10	T.S.	m	0.66	10.5
11	H.Y.	f	0.86	5.1
12	Y.S.	f	0.78	9.1
13	T.S.	f	0.66	7.4
14	Y.F.	f	1.03	7.5
15	S.S.	m	1.01	4.2
16	N.O.	f	0.77	8.1
17	S.N.	m	1.22	11.5
18	Z.F.	m	0.52	6.7
19	I.O.	f	0.45	7.0
20	S.N.	m	0.65	5.6
21	T.S.	m	0.66	3.3
22	M.F.	f	0.64	5.1
23	N.O.	f	0.48	6.9
24	T.S.	m	0.44	5.8
25	M.M.	f	0.70	6.5
26	I.S.	f	0.46	6.4
27	S.O.	m	0.61	5.5
28	C.F.	f	0.46	5.3
29	J.F.	m	0.42	4.8
Average			0.683 ± 0.248	6.76 ± 2.31

Serum ascorbic acid and blood riboflavin levels were determined on 14 men and 15 women in June, 1956. The results are shown in Table 1. The average value of ascorbate was 0.683 ± 0.248 mg per 100 ml serum and that of riboflavin was 6.76 ± 2.31 γ per 100 ml whole blood. Of 29 subjects, 12 were above 0.70 ml per cent of serum ascorbate, and their average blood riboflavin level was 7.33 ± 2.02 γ per 100 ml while that of 17 subjects who were under 0.69 mg per 100 ml of serum ascorbate was 6.36 ± 2.47 γ per 100 ml of blood riboflavin. Fig. 2 shows the correlation. The coefficient of correlation was +0.36 (N=29) and positive correlation between serum acid and blood riboflavin level was significant at the 5 per cent level.

TABLE II SERUM ASCORBIC ACID AND BLOOD RIBOFLAVIN CONCENTRATIONS

	No. of Subjects	Ascorbic Acid Mean Level mg%	Riboflavin Mean Level γ%
Subjects over 0.70 mg % of Ascorbate	12	0.931	7.33 ± 2.02
Subjects under 0.69 mg % of Ascorbate	17	0.506	6.36 ± 2.47
Whole Subjects	29	0.683 ± 0.248	6.76 ± 2.31

Fig. 2. Serum Ascorbic Acid and Blood Riboflavin Concentrations

2) *Ascorbic Acid and Choline in Serum* The investigation were made in the summer and fall of 1954 and the winter of 1955. The serum vitamins analyses were made on 48 subjects. The mean

serum ascorbic acid level was 0.716 mg% and the mean serum choline level was 21.13 mg per 100 ml. The ascorbic acid level in the serum was not related to the serum choline level, as shown by a correlation coefficient of -0.213 (Fig. 3).

Fig. 3. Serum Ascorbic Acid and Serum Choline Concentrations

3) *Ascorbic Acid and Vitamin A in Serum* Assays for the vitamins were made on 34 males and 20 females both selected at random from the farmers

Fig. 4. Serum Ascorbic Acid and Serum Vitamin A Concentrations

in June of 1955. The relationship between ascorbic acid and vitamin A is illustrated in Fig. 4. There was found no relation between the levels of two vitamins (the correlation coefficient was -0.054). The subjects had serum ascorbic acid levels averaging 0.974 ± 0.212 mg% whereas the vitamin A level was 84.4 ± 35.3 I. U. %. The mean vitamin A level of 24 subjects who had ascorbic acid above 1.00 mg% was 88.1 I. U. %, and that of thirty subjects below 0.99 mg% was 81.5 I. U. The vitamin A levels tended to be slightly high in the subjects who showed the high ascorbic acid level, but this difference is not significant.

TABLE III SERUM ASCORBIC ACID AND SERUM VITAMIN A CONCENTRATIONS

	No. of Subjects	Vitamin A Mean Level
Above 1.00 mg% of Ascorbate	24	88.1
Under 0.99 mg% of Ascorbate	30	81.5
Whole Subjects	54	84.4 $\pm$ 35.3

DISCUSSION

The observations on the relationship between ascorbic acid and riboflavin in the body have been previously reported (7-10). They demonstrated that the limitation of ascorbic acid from the diet caused a diminution of riboflavin in the organs of guinea pigs and ascorbic acid contents were decreased in the viscera of rats and dogs in riboflavin deficiency. Stepp (11) suggested that vitamins participating in oxidation-reduction systems such as riboflavin and ascorbic acid can presumably replace each other. It is, therefore, important that the balance of these vitamins is maintained in the body.

Takahashi, a member of our laboratory, performed serum riboflavin determination on the farmers at the same area as was selected by the author, and found that the vitamin level was 1.75 ± 0.56 γ % in the spring, 2.91 ± 1.13 γ % in the summer, 4.92 ± 2.49 γ % in the autumn and 2.05 ± 0.37 γ % in the winter (12). The author also found that the mean level of ascorbic acid was 0.812 mg%, 0.905 mg%, 1.040 mg% and 0.551 mg% in the same order (1). These findings indicate that the seasonal variations in serum ascorbic acid levels are similar to those in serum riboflavin levels.

Kimura, measuring blood pressures on about

seventy farmers at the same area, found that their blood pressures tended to rise in winter (13). In the spring (April) of 1955, Takanohashi et al. (14) carried out the follow-up dietary questionnaire and one-day food intake records and found that daily ascorbic acid and riboflavin intake averaged 46 mg and 0.6 mg respectively.

A positive correlation was obtained between serum ascorbate and blood riboflavin level, whereas there was no correlation between serum ascorbate and serum riboflavin level. A report, recently published by Bessey and his coworkers (15), may explain this reason. They suggested that red blood cell riboflavin content could serve as a reliable measure of riboflavin intake or dietary adequacy in man, and the value of serum level could not represent clearly a measure of nutritive status with respect to this vitamin. Therefore, it is considered that a correlation exists significantly between serum ascorbate and blood riboflavin, which contains red blood cell riboflavin.

Few studies have been made on the effects of ascorbic acid on the choline content of the blood. There is a report that the formation of choline from methionine and ethanolamine or serine in liver preparations was activated by ascorbic acid in the presence of cobalt (16). Moreover, blood ascorbic acid (17) and choline level decrease frequently in hepatic disease. From the facts, it is considered that these vitamins might be related to each other through the liver.

Concerning interrelations between ascorbic acid and vitamin A, there have been several reports with various species of animals and they are suggesting that, when the intake of vitamin A is low, there is a decrease in the vitamin C content of tissues (18). Storvick et al. (19) reported that there was a significant correlation between ascorbic acid and vitamin A in the serum. On the contrary, the data in this paper could not indicate any correlation between them. Further investigation will be needed along this line.

2. RELATIONS OF ASCORBIC ACID CONCENTRATION TO SODIUM, CHOLESTEROL CONCENTRATIONS AND CHOLINESTERASE ACTIVITY IN HUMAN SERUM

It is recently recognized that ascorbic acid has relationships with sodium (20-21) and cholesterol (22-23), and that ascorbic acid (17) and choline-

sterase (24-25) in serum reduced in hepatic diseases. In the present study, therefore, the ascorbic acid, sodium, cholesterol concentrations and cholinesterase activity in the serum were measured and the interrelationships of ascorbic acid to other minor components were studied.

EXPERIMENTAL METHODS

Serum Sodium Concentration Immediately after blood was taken from the cubital vein by a syringe which was dried after washing with distilled water, it was transferred into a spitz tube and incubated for twenty-five minutes in water-bath at 37°C. The serum was, then, separated by centrifugation and 1.0 ml of it was taken up into 50 ml messflask which was filled up with water. The measurement was performed with Lange's flame-photometer according to Tasaka et al (26).

Serum Total Cholesterol Concentration Total cholesterol level in the serum was determined by the method of Schoenheimer-Sperry using digitonin (27).

Serum Cholinesterase Activity The measurement was carried out by the modified method (28) of Takahashi and Shibata (29). Immediately after collecting blood, the serum was separated and 25 mg acetylcholine was added to 0.1 ml of the serum. Activity was expressed in terms of changes (Δ pH) in acidity result from liberation of acetic acid from the acetylcholine substrate.

EXPERIMENTAL RESULTS

Ascorbic Acid and Sodium The values of ascorbic acid and sodium of serum, obtained from thirty-two subjects ranging from 33 to 55 years of age, were measured in August of 1956, and the results are shown in Fig. 5. The average value of serum ascorbic acid in the whole subjects was 0.853 ± 0.259 mg% and that of sodium was 142.1 ± 4.3 mEq/l. The correlation between serum ascorbic acid level and serum sodium content was negative; $r = -0.39$, which was slightly significant at the 10 per cent level.

Ascorbic Acid and Cholesterol Chemical determinations of ascorbic acid and cholesterol levels of serum from twenty-five person ranging from 27 to 55 years of age were made, and the correlation diagram is illustrated in Fig. 6. The average value of serum vitamin was 0.53 ± 0.169 mg% and serum cholesterol was 140.6 ± 37.9 mg%. The coefficient

Fig. 5. Serum Ascorbic Acid and Serum Sodium Concentrations

Fig. 6. Serum Ascorbic Acid and Serum Cholesterol Concentrations

of correlation was -0.067 and this was not significant.

Ascorbic Acid and Cholinesterase Ascorbic acid level and cholinesterase activity in the serum were measured on 38 subjects in February, 1955. The subjects who were positive in urine-urobilinogen-reaction were omitted, because the serum cholinesterase is influenced by the condition of hepatic disease. The results are shown in Table 4 and Fig. 7. The mean level of serum ascorbic acid was 0.543 ± 0.139

mg% and that of serum cholinesterase, 1.169 ± 0.174 Δ pH. The average of serum cholinesterase activities of 28 hypertensive patients above 150 mm Hg in systolic and 90 mmHg in diastolic blood pressure was 1.118 ± 0.149 Δ pH, while that of ten

Fig. 7. Serum Ascorbic Acid Concentrations and Cholinesterase Activities

TABLE IV SERUM ASCORBIC ACID CONCENTRATIONS AND CHOLINESTERASE ACTIVITIES

No.	Name	age	sex	Blood Pressure mmHg	Ascorbic Acid mg%	Cholinesterase Δ pH
1	H.O.	55	♀	182 ~ 120	0.72	0.99
2	Z.F.	58	♂	140 ~ 98	0.50	1.29
3	Y.F.	35	♂	134 ~ 56	0.52	1.28
4	S.S.	69	♀	242 ~ 118	0.40	1.34
5	F.S.	30	♀	172 ~ 108	0.38	1.14
6	C.F.	44	♀	198 ~ 118	0.50	1.09
7	C.Y.	30	♂	160 ~ 90	0.62	1.24
8	Z.S.	43	♂	128 ~ 80	0.71	1.19
9	W.N.	50	♂	124 ~ 84	0.50	1.28
10	M.Y.	74	♂	158 ~ 86	0.39	0.89
11	T.O.	21	♀	146 ~ 92	0.66	1.13
12	T.S.	43	♂	148 ~ 88	0.45	1.36
13	Y.S.	48	♀	196 ~ 100	0.56	1.01
14	T.F.	35	♀	154 ~ 105	0.53	1.29
15	H.K.	55	♂	128 ~ 82	0.45	0.74
16	T.Y.	55	♂	154 ~ 86	0.49	1.08
17	Y.F.	49	♂	125 ~ 82	0.90	1.39
18	C.F.	41	♂	160 ~ 100	0.50	0.97
19	K.F.	34	♂	134 ~ 86	0.60	1.42
20	L.O.	38	♀	154 ~ 100	0.42	1.49
21	Y.F.	40	♂	136 ~ 88	0.35	0.99
22	C.Y.	49	♂	158 ~ 84	0.52	1.07
23	J.F.	63	♂	102 ~ 132	0.50	1.35
24	M.Y.	54	♂	164 ~ 88	0.50	1.32
25	Z.M.	43	♂	150 ~ 88	0.61	0.91
26	I.F.	43	♂	154 ~ 92	0.47	1.20
27	T.S.	51	♀	224 ~ 112	0.47	1.19
28	S.N.	35	♂	128 ~ 82	0.54	1.01
29	K.F.	58	♂	216 ~ 70	0.49	1.10
30	L.F.	53	♀	138 ~ 106	0.45	1.43
31	M.Y.	69	♂	172 ~ 110	0.47	1.05
32	M.F.	58	♀	128 ~ 82	0.64	1.51
33	H.O.	40	♀	122 ~ 94	0.72	1.20
34	T.M.	45	♀	145 ~ 96	0.57	0.87
35	M.N.	53	♀	155 ~ 98	0.58	1.14
36	Z.F.	62	♂	150 ~ 98	0.53	1.20
37	M.M.	37	♀	168 ~ 98	1.00	1.07
38	I.S.	69	♂	194 ~ 128	0.71	1.15
Average					0.543 ± 0.159	1.169 ± 0.174

normotensive subjects was $1.213 \pm 0.221 \triangle \text{pH}$.

DISCUSSION

A number of investigations have indicated that high levels of intake of sodium chloride are important to the development of hypertension in animals (30-31) and other observations have revealed the evidences that sodium withdrawal in the diet might influence the reduction of blood pressure in hypertensive patients (32).

The inhabitants of the place where this experiment was performed, in which mortality from apoplexy is high, take a high level of salt throughout the year, and many of them take more than 20 grams of salt daily even when they do not perspire. Koyanagi and Ota (the latter is a member of our laboratory) measured the concentration of salt in the Miso-soup eaten by the inhabitants of the two villages and found that the concentration was high (33). The author, however, observed that the blood pressures of the workers at the melting furnace were not elevated, even though they had taken a large amount of salt during the last 4 to 23 year period (34). The author assumed, in consequence, that even though a large amount of salt is taken by persons with normal renal and adrenal functions, they might not show high blood pressures if they perspire profusely. Doi observed that excessive intake of sodium chloride —20 grams per day— increases a basal metabolic rate and blood pressure and decreases plasma ascorbic acid level in man (19). This phenomenon, however, was not observed in the simultaneous administration of ascorbic acid (50 mg daily) or in summer, and an increase of plasma ascorbic acid level was found by the restriction of salt intake (3g per day). The author has recognized that there is a significant negative correlativity between ascorbic acid level and sodium value in the serum. This tendency must be revealed more clearly in winter, so a further investigation will be needed throughout the year.

Some reports (21-22) concerning ascorbic acid and serum cholesterol have been published and have suggested that a continued use of ascorbic acid to patients with hypertony and atherosclerosis lowered the cholesterolemia, and ascorbic acid reduced blood cholesterol and inhibited the development of fat deposits in the aorta in the rabbits with experimental cholesterol atherosclerosis. On the contrary, there is a report that administration of ascorbic acid

resulted in a rise of serum cholesterol level in animals (35), and Gillum et al. (36), in their study on older women, found a slight positive relationship between serum cholesterol and serum ascorbic acid.

Cholesterol is considered to be a precursor of steroid hormones, and presumably cholesterol and ascorbic acid are closely related to the production of these hormones in adrenals. Ascorbic acid possibly plays a role in normalizing the blood cholesterol level. A further investigation is made to ascertain the influence of the administration of ascorbic acid on the serum cholesterol of rats with high-salt diets. The results are to be reported in the future.

Ascorbic acid restores or increases serum cholinesterase activity (37-38) and cholinesterase in scorbutic tissues is decreased (39). Pantschenko-Jurewicz and Kraut (40) showed that ascorbic acid had a character of coenzyme for liver esterase, and therefore, their findings may be the reason why the vitamin increases the enzyme activity.

SUMMARY

Chemical analyses of vitamins, sodium, cholesterol concentrations and cholinesterase activity in serum or blood of the farmers in the rice-producing districts of central Iwate-Ken were made and the interrelationships of ascorbic acid to the vitamins and the minor subjects were studied. The results are as follows:

- 1) A significant correlation between the serum ascorbic acid level and the blood riboflavin level was found, while there was no relationship between the vitamin and serum riboflavin level.
- 2) There was no interrelationship of ascorbic acid level to choline and vitamin A levels in the serum.
- 3) A significant negative correlation between ascorbic acid level and sodium concentration in serum was found.
- 4) Correlations of ascorbic acid level to cholesterol level and cholinesterase activity were not significant.

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