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Response of Guinea Pigs to Ascorbic Acid II. Serum protein, Albumin and Globulin

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THIS work was performed to study the role of ascorbic acid supplementation on the serum protein, albumin and globulin of guinea pigs. The experimental animals were given different levels of ascorbic acid and housed under normal and heat stress conditions. Blood samples were withdrawn after one, three and five weeks for determining serum protein, albumin and globulin. Total serum protein level was significantly decreased as a result of ascorbic acid deficiency. Exposure to heat stress enhanced this negative effect. Serum albumin followed the same pattern of the total protein in response to ascorbic acid deficiency. Serum globulin tended to increase in the group of animals fed on the scorbutogenic diet. When the scorbutogenic diet was supplemented with large doses of ascorbic acid the biological parameters were close to normal.

The guinea pig is a valuable animal for nutritional research because of its unusually high requirement for certain vitamins and amino acids. Among the commonly used laboratory animals, only it and the primates require a dietary source of ascorbic acid. Considerable progress has been made in determining its nutritional requirements, and fuller knowledge should increase its usefulness as an experimental animal.

Sevkovic *et al.* (1976) reported that vitamin C was shown to be of great importance for reducing the pale, soft exudative defect and muscle degeneration in pork. The effect differed according to the method by which it was given and biological factors connected with the rearing system. Denvyatka and Yatsyna (1978) found that vitamin C in blood decreased in rabbits not given ascorbic acid supplement and also decreased in the adrenals, kidneys, spleen and other organs. In groups given the supplements, vitamin C in blood and organs generally increased. Alekseev (1980) demonstrated that the pigs given vitamin C had greater blood contents of ascorbic acid.

Material and Methods

Sixty four guinea pigs 3 weeks old, body weight ranged between 170 and 195 g were experimented on. The animals were housed in plastic 40 $\times$ 30cm cages, two in each one.

The experimental animals were divided into 6 groups and fed on a scorbutogenic diet (Hughes and Hurley, 1969) at a rate of 8 g/100 g body weight. Water was available freely throughout the experimental period and 0.05 ml of cod liver oil was given weekly to each animal. The composition of the scorbutogenic diet was as follows :-

Wheat bran	35.0%
Clover hay	37.0%
Dried whole milk	10.0%
Dried skim milk	10.0%
Dried yeast	6.5%
Mineralized salt	1.0%
Magnesium oxide	0.5%

The aforementioned diet was analyzed according to A.O.A.C. in (1975), the chemical composition was as follows :-

Moisture	8.20%
Crude protein	16.70%
Ether extract	4.30%
Crude fiber	5.23%
N-free extract	60.76%
Ash	4.81%

The six groups of animals were intramuscularly injected with ascorbic acid and located in the normal and high constant temperatures as follows :-

Treatments	Groups					
	1st	2nd	3rd	4th	5th	6th
Number of animals	14	10	10	10	10	10
Supplemented ascorbic acid/100 g body weight .	0.5 mg	0.5mg	—	—	5mg	5mg
Housing temperature, °C . . .	19—22	35	19—22	35	19—22	35

1. The level 0.5 mg/100 g body weight is considered the daily requirements of vitamin C for guinea pigs (NAS-NRC, 1972).
2. The temperatu 19-22 is considered the optimum for guinea pigs (Laue-Petter and Porter, 1963).
3. The temperature 35 °C causes stress for guinea pigs (Zeman *et al.*, 1966).
4. Ascorbic acid level of 5 mg/100 g body weight equals 10 fold requirements.

Blood collection was taken from the orbital sinus of the animals (Schemer, 1967). The samples were withdrawn after one week from the beginning of the experiment and then every two weeks until the termination of the trial (5 weeks).

Serum samples were analyzed for total protein using the Buret method Gornall *et al.* 1949), albumin by the colorimetric method using bromocresol green (Doumas *et al.* 1971), globulin by the difference between total protein and albumin (Reinhold, 1953).

The data were statistically analyzed after Steel and Torrie (1960).

Results and Discussion

Effect of ascorbic acid deficiency on total serum protein of Guinea Pigs

The total protein in the serum of guinea pigs fed the scorbutogenic diet was found to be less than that of the control animals. After 7 days, its value 5·6 compared to 5·8 g /100 ml for the scorbutic and control animals respectively. The difference was more clear after 3 weeks, the corresponding values were 5·3 and 5·9 g /100 respectively. The same difference was observed at the end of the experiment (35th day), there was a drop of 0·6 g/100 ml from the control group (Table 1).

Total serum protein for the animals received 10 folds the daily requirements of ascorbic acid was nearly equal to that of the controls and higher that of the scorbutic animals. The same criteria was found for the animals exposed to heat.

Analysis of variance showed that the serum protein was significantly affected as a result of nutrition and temperature (Table 1).

The total serum protein of scorbutic animals housed in the normal environment declined from 5·6 to 5·2 g / 100 ml throughout the experimental period. Animals exposed to heat did not show appreciable change in total serum protein.

Animals received normal daily reqirements of vitamin C did not show any change in total serum protein under normal temperature, meanwhile a slight increase was observed in those animals exposed to heat.

For the animals recieved 10 folds the daily requirements of vitamin C and housed in the normal environment, the total serum protein increased from 5·3 to 5·6 g/100 ml with a percentage of 5·7%. For the guinea pigs exposed to heat, the same component increased from 4·9 to 5·5 g/100 ml with a percentage of 12·2%.

TABLE 1. Effect of giving ascorbic acid to guinea pigs on serum protein under normal and heat stress conditions.

Intervals from the start of the experiment	Mean serum protein g/100 ml					
	19 — 22 °C			35 °C		
	Scorbutogenic diet + 0.05 mg ascorbic acid/ 100 g B.W.	Scorbutogenic diet	Scorbutogenic diet + 5mg ascorbic acid 100 g B.W	Scorbutogenic diet+0.05 mg ascorbic acid/ 100 g B.W.	Scorbuto-genic diet	Scorbuto-genic diet+5 mg ascorbic acid/ 100 g B.W.
7 days	5.8 ± 0.16	5.6 ± 0.22	5.3 ± 0.20	5.4 ± 0.19	5.1±0.22	4.9 ± 0.20
21 days	5.9 ± 0.16	5.3 ± 0.22	5.9 ± 0.20	5.8 ± 0.19	5.2±0.22	5.3 ± 0.20
35 days	5.8 ± 0.17	5.2 ± 0.25	5.6 ± 0.20	5.7 ± 0.20	5.1±0.25	5.5 ± 0.23
Mean	5.8	5.4	5.6	5.6	5.1	5.2

	F value
Between nutrition	9.90*
Between temperatures	10.30*
Between periods	2.61

* $p < 0.01$

The mean level of serum protein of guinea pigs housed in the normal temperature was found to be 5.5 versus to 5.3 g/100 ml for the animals exposed to heat stress.

The findings obtained as regard to serum protein coincide with those recorded by Mikalanskaite (1959). In this connection Cesh and Bhattacharya (1966) reported a greater total protein levels in animals received high level of vitamin C.

Effect of ascorbic acid deficiency on serum albumin of Guinea Pigs

Ascorbic acid deficiency was found to decrease the serum albumin compared to the control guinea pigs (Table 2). After 7 days of vitamin C deprivation, the serum albumin was 2·1 versus to 3.6 g/100 ml for the control animals, constituting a drop of 71·3%. However, at the termination of the experiment the drop was less, *i.e.* 18·5%.

For the animals received 10 folds of the daily reqirements of ascorbic acid, the values obtained were generally higher than the scorbutic group, starting from the third week they recorded higher levels than the control animals.

The same observation was noticed for the Guinea pigs exposed to heat stress.

The serum albumin increased in the scorbutic animals from 2·1 to 2·7 g/100 ml with a percentage of 28%. In those animals exposed to heat the increase in serum albumin was less, from 2·4 to 2·6 g/100 ml, *i.e.*, 8·3%. The same trend was noticed in the guinea pigs recieved 10 folds the daily requriements of vitamin C, the serum albumin increased from 2·4 to 3·4 g/100 ml with a percentage of 42 %. The increase was less in those animals exposed to heat, the serum albumin increased from 2·6 to 2·9 g/100 ml with a percentage of 11·5%.

It can be noticed that the mean level of serum albumin of the guinea pigs housed at normal temperature and heat stress were similar (Table 2).

Analysis of variance for the effect of ascorbic acid intake on the serum albumin was significant statistically.

The results concerning the total protein and serum albumin can be explained on the basis of the biological activities of vitamin C. This spectrum of activity extend down to the production of the energy essential to protein anabolism according to Kern and Racker (1954). They were able to prove that the oxidation of reduced NADH was stimulated by the addition of ascorbric acid. Furthermore, Kersten *et al.* (1958) used an enzyme system isolated from superarenal microsomes for the oxidation of NADH. They found that neither dehydro - ascrobic acid nor glutathione could replace ascrobic acid. They considered ascrobic acid free radical as an intermediary electron acceptor in the system.

A further step in the biosynthesis of proteins is the availability of essential amino acids needed for the formation of protein molecule. Schonheyder and Lyndlye (1962) demonstrated that there is derangement of the free amino acids in the serum of scorbutic guinea pigs. It was found that the blood levels of threonine, valine, lysine and arginine were increased in scorbutic animals indicating impaired tissue utilization to form protein molecules. Even the enzymes responsible for the metabolism of protein were found to be affected by the blood levels of ascorbic acid.

Cesh and Bhattacharya (1966) demonstrated that the levels of xeanthin oxidase activity in the liver and aspartate aminotransferase and alanine aminotransferase activities in the liver, heart, kidney and plasma increased with increasing concentration of vitamin C in the diet.

Effect of ascorbic acid deficiency on serum globulin of Guinea Pigs.

Table 3 shows that the mean of serum globulin of the scorbutic guinea pigs was higher than that of the control animals. The serum globulin of the scorbutic animals was higher than that of the controls at one week (a percentage difference of 66·6 %) compared to 5·2 % after 3 weeks. A lower value was noticed near the end of the experiment (2·5 versus to 2·7 g/100 ml).

TABLE 2. Effect of giving ascorbic acid to guinea pigs on serum albumin under normal and heat stress conditions.

Intervals from the start of the experiment	Mean serum albumin g/100 ml					
	19 — 22 °C			35 °C		
	Scorbutogenic diet + 0.05 mg ascorbic acid / 100 g B.W.	Scorbutogenic diet	Scorbutogenic diet + 5 mg ascorbic acid/ 100 g B.W.	Scorbutogenic diet + 0.05 mg ascorbic acid/ 100 g B.W.	Scorbuto-genic diet	Scorbuto-genic diet + 5 mg ascorbic acid/ 100 g B.W.
7 days	3.6 ± 0.16	2.1 ± 0.21	2.4 ± 0.19	3.5 ± 0.18	2.4±0.21	2.6±0.19
21 days	3.0 ± 0.16	2.3 ± 0.21	3.3 ± 0.19	3.1 ± 0.18	2.8±0.21	3.2±0.19
35 days	3.2 ± 0.16	2.7 ± 0.24	3.4 ± 0.19	3.1 ± 0.19	2.6±0.24	2.9±0.2z
Mean	3.3	2.4	3.0	3.2	2.6	2.9

	F value
Between nutrition	23.79*
Between temperatures	0.17
Between periods	1.33

* $P < 0.01$

The serum globulin of the scorbutic animals exposed to heat behaved completely irregularly.

For the animals received 10 folds the daily requirements of vitamin C, the levels of serum globulin were less than those of the scorbutic animals when compared to the control ones.

Furthermore, the same irregularity was observed in those guinea pigs exposed to heat when compared to the control animals.

After one week, the mean of the serum globulin of the scorbutic animals at normal temperature was 3·5 versus to 2·7 g/100 ml for the animals exposed to heat. However, it declined to 2·5 g/100 ml at the normal and heat stress conditions, indicating that heat decreased the drop of serum globulin observed.

The same finding was observed in the guinea pigs received 10 folds the daily requirements of ascorbic acid where there was a drop from 2·9 to 2·2 g/100 ml with a percentage of 31·8 % versus to an increase of 8·6 % to those animals exposed to heat (2·3 to 2·5 g/100 ml).

TABLE 3. Effect of giving ascorbic acid to guinea pigs to serum globulin under normal and heat stress conditions.

Intervals from the start of the expeiment	Mean serum globulin g/100 ml					
	19—22 °C			35° C		
	Scorbutogenic diet+0.05mg ascorbic acid/100 g B.W.	Scorbutogenic diet	Scorbutogenic diet +5 mg ascorbic acid/100 g B.W.	Scorbutogenic diet+0.05mg ascorbic acid/100 g B.W.	Scorbutogenic diet	Scorbutogenic diet + 5mg ascorbic acid/100 g B.W.
7 days	2.1 ± 0.18	3.5 ± 0.24	2.9 ± 0.22	2.1 ± 0.21	2.7±0.24	2.3 ± 0.22
21 days	2.9 ± 0.18	3.1 ± 0.24	2.6 ± 0.22	2.8 ± 0.21	2.4±0.24	2.2 ± 0.22
35 days	2.7 ± 0.19	2.5 ± 0.28	2.2 ± 0.22	2.5 ± 0.22	2.5±0.28	2.5 ± 0.25
Mean	2.6	3.0	2.6	2.5	2.5	2.3

	F value
Between nutrition	3.17*
Between temperatures	5.16*
Between periods	1.12

* $P < 0.01$

Analysis of variance showed that variations of ascorbic acid intake and temperature changes on serum globulin showed significant effect (Table 3).

An approach to the problem of deragement of protein metabolism in deficiency of ascorbic acid could be adopted through the fact that protein metabolism is under the influence of hormonal functions. Many hormones are known to affect the protein metabolism as growth hormones, ACTH, thyroid hormones are known to exert their action on the living cell through a second mesenger or mediator, the cyclic AMP. The above mentioned concept was first proposed and established by Sutherland (1971).

Some reports appeared suggesting a possible role of ascorbic acid on the cyclic AMP and hence on hormonal function. Earp *et al.* (1970) reported that the adrenal cortical cyclic AMP produced a similar effect to that of ACTH on ascorbic depletion. Furthermore, ascorbate was found to assist the production of adrenalin from dopamine through C. AMP (Lewin, 1976)

It is thus possible that the deficiency of ascorbic acid can result in affection of the hormonal functions responsible for protein metablolism through cyclic AMP. This concept can explain the disturbance in the levels of serum albumin and globulin observed in this work.

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تأثير تغذية خنازير غينيا بحمض الاسكوربيك ((فيتامين ج))
٢ • مستوى البروتين والألبيومين والجلوبيولين فى سيرم الدم

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أجرى هذا البحث لدراسة تأثير الامداد بحمض الاسكوربيك « فيتامين ج » على مستوى البروتين والالبيومين والجلوبيولين فى سيرم الدم لحيوانات خنازير غينيا • أعطيت حيوانات التجارب مستويات مختلفة من حمض الاسكوربيك وتم اسكانها تحت كل من الظروف العادية والحرارة المرتفعة •

أخذت عينات دم على فترات أسبوع ، ثلاثة ، خمسة أسابيع لتقدير البروتين والألبيومين والجلوبيولين فى سيرم الدم • انخفض البروتين الكلى معنويا فى سيرم الدم نتيجة انخفاض مستوى حمض الاسكوربيك فى الغذاء • كان لظروف ارتفاع درجة الحرارة تأثيرا مساعدا على ذلك التأثير السلبى • ولقد أخذ البيومين السيرم نفس اتجاه البروتين الكلى فيما يتعلق بمستوى حمض الاسكوربيك فى الغذاء • وفيما يختص بجلوبيولين سيرم الدم فلقد زاد فى مجموعة الحيوانات المغذاة على مستوى منخفض من حمض الاسكوربيك • عندما أجرى تعزيز الغذاء المنخفض فى حمض الاستكوربيك بجرعة عالية منه فان المدلولات البيولوجية لحيوانات التجارب عادت لحالتها العادية •