

Response of Guinea Pigs to Ascorbic Acid I. Growth, Serum Ascorbic Acid and Total Lipids

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WHEN THE Guinea Pigs received a diet deficient in ascorbic acid their body weight was significantly lower than that of normally fed animals. Exposure of the animals to heat stress exaggerated the effect of ascorbic acid deficiency. The animals fed on scorbutogenic diet suffered from severe symptoms of scurvy and low serum ascorbic acid level. The animals in this group were very weak and showed petechial hemorrhage. The mean total lipids level in the serum increased significantly in association with ascorbic acid deprivation, but was not affected by heat stress. It is very likely that ascorbic acid plays an important role in mammalian growth. When the scorbutogenic diet was supplemented with large doses of ascorbic acid, the biological parameters were close to normal.

Ascorbic acid is the vitamin that was designated as water-soluble C, or the antiscorbutic factor. The metabolic need appears to be a general one among species, but a dietary need is limited to man, the guinea pig, and the monkey. The ability of the pig to meet its metabolic needs from birth to maturity by synthesis has been demonstrated by Braude *et al.* (1950). Denvyatka and Yatsyna (1978) found that vitamin C in blood decreased in rabbits not given ascorbic acid supplement and not given ascorbic acid supplement and exposed to excessive sunlight from between 0.77 and 0.94 to between 0.53 and 0.64 mg/100 ml and also decreased in the adrenals, kidneys, spleen and other organs. In groups given the supplements, vitamin C in blood and organs generally increased. Yen and Pond (1981) stated that dietary vitamin C supplementation to pigs improved daily gain and daily feed intake without altering gain to feed ratio. Plasma ascorbic acid concentration decreased during the 4 weeks after weaning in pigs not given supplemental vitamin C but not in those received. Simple correlation indicated a positive ($p < 0.05$) relation between plasma ascorbic acid concentration and gain.

Material and Methods

Sixty four male guinea pigs 3 weeks old, body weight ranged between 170 and 195 g were experimented on. The animals were housed in plastic 40 × 30 cm 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	0.5 mg	—	—	5 mg	5 mg
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 temperature 19-22° is considered the optimum for guinea pigs (Laue-Petter and Porter, 1963).
3. The temperature 35° causes stress for guinea pigs (Zeman *et al.*, 1966).
4. Ascorbic acid level of 5 mg/100 g body weight equals 10 fold requirements.

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The weighing of the animals was performed before feeding at the commencement of the experiment and weekly thereafter. Blood collection was 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 ascorbic acid using the method of Roe (1966) and total lipids using the procedure of Knight *et al.* (1972).

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

Results and Discussion

Effect of ascorbic acid deficiency on the body weight of Guinea pigs

Table 1 shows that at the termination of the experimental period (35 days), the mean weight of the scorbutic guinea pigs showed a more or less a steady state compared to a further gain in the control group. The overall increase in weight of scorbutic animals was found to be 6.8 % versus to 66.6 % for the controls.

The amount of weight gain for the animals received 10 folds the daily requirements of scorbutic acid was below than that of the control group. The guinea pigs received Mega dose of vitamin C increased by 36.2 % versus to 66.6 % for the control animals.

The fact that guinea pigs loose weight when fed scorbutogenic diet have been previously reported by some authors. Odumosu and Wilson (1973) demonstrated that the animals start to loose weight on scorbutogenic diet from the 24th day of the experiment and by the 36th day they were all died. Barnes and Kodicek (1972) reported that loss of weight started after 10 days of ascorbic acid deprivation, death followed 3-4 weeks after the introduction of the ascorbic acid free diet.

Lewin (1976) reported that the synthesis of certain amino acids containing disulphide bonds is dependent on ascorbic acid. Schonheyder (1962) found that the scorbutic animals showed a disturbance in both the serum amino acids and the level of enzyme necessary for the biosynthesis of proteins. The presence of high concentration of ascorbic acid in the pancreas (Yavorsky *et al.*, 1934) can confirm the effect of this vitamin on tissue anabolism. NRC (1972) cited that the gross effects produced by the absence of ascorbic acid from the diet on guinea pigs are anorexia, retarded growth and death. Burns *et al.* (1956) and Odumosu and Wilson (1973) reported that guinea pigs are unable to synthesis their own ascorbic acid.

Therefore, the inability of guinea pigs to synthesis their own ascorbic acid, the wide range of metabolic activities in which ascorbic acid participates can explain the loss of weight and death of these animals on ascorbic acid free diet.

It can be stressed that the weight is rather a crude parameter in a sense that it reflects the overall metabolic derangement occurring at the biochemical level.

In the present work, it was found that the values obtained concerning the variations of body weight according to the changes in the amount of ascorbic acid intake, the temperature changes and the duration of the experiment were significant statistically (Table 1).

Effect of heat stress on body weight of Guinea pigs

The average body weight of the guinea pig housed in the normal conditions was increased throughout the experimental period (35 days) with the amount of 66.6%. On the other hand, the corresponding gain for the animals of the heated group was 56.3%. It can be concluded that the increase in weight in those animals exposed to heat below than those housed at normal conditions. Taking into consideration that both groups received their daily requirements of vitamin C, it could be deduced that this difference is due to decrease of the appetite of the animals exposed to heat. It could not be attributed to an increase of vitamin C requirements due to this stressful situation. Animals received 10 folds the daily requirements of ascorbic acid showed also a difference in the percentage of weight gain between those housed in the two different environmental conditions.

However, the effect of heat on the weight gain of guinea pigs was more effective in the group of animals fed the scorbutogenic diet. They showed a loss of body weight with a percentage of 5.5%. On the other hand, animals fed the same level of vitamin but housed in the normal conditions gained 6.6% of their initial weight. This can be explained on the basis of increased vitamin C requirements by the animals exposed to heat.

In this connection Harris *et al.*, (1953) demonstrated an effect of ascorbic acid depletion on the adrenal gland, a one that has an effective role in stress conditions. Morbid anatomical changes were noticed in the adrenals of scorbutic guinea pigs. These glands are hypertrophied in scurvy.

Glucocorticoid levels were higher than normal in the laboratory animals developing hyperthermia in response to environmental heat (Kotby and Johnson, 1967). Young growing animals were more responsive to heat stress as compared to mature animals as was reflected in the elevated circulating glucocorticoids (Kotby *et al.*, 1967). Therefore, body weight loss of guinea pigs in the present study may be due to gluconeogenic effect of higher circulating glucocorticoid levels. Another possibility for such body weight decrease in the heat stressed scorbutic animals in this study, is the inhibition of catecholamines synthesis, since ascorbic acid was reported to participate in the synthesis of catecholamines (Blumberg *et al.*, 1965 and Lewin, 1976). If this is true, then ascorbic acid is of particular importance in adrenal gland junctional activities.

Effect of ascorbic acid deficiency on its level in the serum of Guinea Pigs

The serum ascorbic acid of the animals received scorbutogenic diet decreased from 0.22 to 0.08 mg/100 ml with a mean of 0.16 mg/100 ml, compared to a means of 0.29 and 0.34 mg/100 ml for the control and the group given 10 folds daily requirements of ascorbic acid respectively (Table 2).

More severe drop in the serum ascorbic acid was noticed in the scorbutic guinea pigs exposed to heat, the value reached 0.03 mg/100 ml near the termination of the experiment.

The scorbic acid intake and the duration of the experiment were found to affect the serum ascorbic acid significantly.

It can be concluded that heat stress increases the requirements of tissues for ascorbic acid, since no exogenous or endogenous supplies are available, the level of ascorbic acid drops markedly. Odumosu and Wilson (1973) reported that the serum ascorbic acid in these animals reached a minimum level on the 30th day. Devyatka and Yatsyna (1978) found that vitamin C in blood decreased in rabbits not given ascorbic acid supplement and exposed to excessive sunlight from between 0.77 and 0.94 to between 0.53 and 0.64 mg/100 ml and also decreased in the adrenals, kidneys, spleen and other organs. In groups given the supplements, vitamin C in blood and organs generally decreased.

Effect of ascorbic acid deficiency on serum lipids of Guinea pigs

The deprivation of vitamin C was found to affect the serum lipids markedly (Table 3). The serum lipids of the scorbutic animals were higher than that of the control group. However, the difference appeared only after 21 days, the rise was 43.9%. Furthermore, the percentage of increase in the serum lipids for the control group throughout the experiment was 44% versus to 53% for the scorbutic guinea pigs. For those animals received 10 folds the daily requirements of ascorbic acid, the serum lipids were below the control or slightly above it.

A marked difference in serum lipids was observed in scorbutic animals under heat stress compared to those at normal temperature. The serum lipids were increased in animals at optimum temperature with a percentage of 53.3% versus to 173% for those exposed to heat. This difference can be explained on the basis of increased requirements of ascorbic acid by the animals exposed to heat. Ascorbic acid being involved in lipids metabolism, its depletion under stress exaggerates their metabolic disturbance observed in scorbutic animals.

Analysis of variance showed that the change in ascorbic acid intake and duration of the experiment altered the total serum lipids significantly under the normal and heat stress conditions (Table 3).

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

Intervals from the start of the experiment	Mean body weight (g)					
	19 - 22°		35°		35°	
	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.	Scorbutogenic diet	Scorbutogenic diet + 5 mg ascorbic acid/100 g B.W.
0 days	183.14 ± 8.89	173.10 ± 10.51	195.00 ± 10.51	180.00 ± 10.51	183.00 ± 10.51	165.60 ± 10.51
7 days	184.00 ± 8.89	163.00 ± 11.76	193.00 ± 11.08	184.20 ± 10.51	180.00 ± 11.76	160.78 ± 11.08
14 days	218.57 ± 8.89	196.62 ± 11.76	225.77 ± 11.08	198.90 ± 10.51	173.50 ± 11.76	198.40 ± 11.08
21 days	251.70 ± 8.89	193.62 ± 11.76	236.22 ± 11.08	222.90 ± 10.51	176.10 ± 11.76	200.30 ± 11.08
28 days	278.40 ± 8.89	182.37 ± 11.76	236.44 ± 11.08	250.70 ± 10.51	169.00 ± 11.76	216.40 ± 11.08
35 days	305.07 ± 9.22	184.83 ± 13.57	265.55 ± 11.08	281.33 ± 11.08	173.00 ± 13.57	208.10 ± 12.57
Mean	236.8	182.3	228.7	219.7	175.8	191.6

F value

72.59*

43.79*

36.92*

Between nutrition

Between temperatures

Between periods

* P < 0.01

TABLE 2. Effect of giving ascorbic acid to guinea pigs on its level in the serum under normal and heat stress conditions.

Intervals from the start of the experiment	Ascorbic acid level in the serum mg/100 ml					
	19 — 22°			35°		
	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.	Scorbutogenic diet	Scorbutogenic diet + 5 mg ascorbic acid/100 g B.W.
7 days	0.32 ± 0.013	0.22 ± 0.018	0.32 ± 0.018	0.30 ± 0.016	0.21 ± 0.018	0.29 ± 0.018
21 days	0.28 ± 0.013	0.18 ± 0.018	0.37 ± 0.018	0.31 ± 0.017	0.19 ± 0.018	0.36 ± 0.018
35 days	0.28 ± 0.013	0.08 ± 0.020	0.32 ± 0.18	0.31 ± 0.017	0.03 ± 0.020	0.32 ± 0.019
Mean	0.29	0.16	0.34	0.31	0.14	0.32

Fvalue
 Between nutrition 45.58*
 Between temperatures 0.79
 Between periods 4.85*
 P < 0.01

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

Intervals from the start of the experiment	Mean total lipids level in the serum mg/100 ml							
	19 — 22°				35°			
	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 + 0.05mg ascorbic acid/100 g B.W.	Scorbutogenic diet	Scorbutogenic diet + 0.05mg ascorbic acid/100 g B.W.	Scorbutogenic diet + 5 mg ascorbic acid/100 g B.W.	Scorbutogenic diet
7 days	114.42 ± 9.82	113.30 ± 12.99	80.03 ± 12.25	115.69 ± 11.62	83.68 ± 12.99	87.98 ± 12.25		
21 days	110.17 ± 9.82	158.60 ± 12.99	123.35 ± 12.25	125.40 ± 11.62	150.45 ± 12.99	97.92 ± 12.25		
35 days	164.92 ± 10.20	174.00 ± 15.00	169.50 ± 12.25	166.66 ± 12.25	228.80 ± 15.00	132.51 ± 13.89		
Mean	129.84	148.63	124.29	135.92	154.30	106.74		

F-value
 Between nutrition 9.38*
 Between temperatures 0.44
 Between periods 48.23*

The results revealed that vitamin C deficiency resulted in elevated serum lipids and vice versa.

In this connection Banerjee and Bandyopadhyay (1963) reported that scorbutic monkeys showed increased level of plasma triglycerides and free fatty acids and increased total B-lipoprotein. All of these effects were removed by ascorbic acid administration with the exception of total plasma cholesterol and B-lipoprotein cholesterol which remained low. Gushlait *et al.* (1963) observed lower oxidation of unsaturated fatty acids by several tissues of scorbutic guinea pigs. High dose of ascorbic acid depressed alimentary hypercholesterolaemia in rabbits and rats and exerted a protective effect as regards to experimental arteriosclerosis (Mjasnikov, 1958 and Sokolff *et al.*, 1967).

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قائمة تغذية خنازير غينيا بحمض الاسكوربيك «فيتامين ج» والتنمية ومستوى حمض الاسكوربيك والدهون في سيريوم الدم

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للبحوث بالدقى ، مصر

عندما غذيت خنازير غينيا على غذاء فقير في حمض الاسكوربيك «فيتامين ج» حدث انخفاض معنوي لوزن الجسم بالمقارنة بالحيوانات في مجموعة المقارنة . الحيوانات المغذاة على غذاء خالي من حمض الاسكوربيك ظهرت عليها أعراض حادة لمرض الاسقربوط إضافة الى انخفاض مستوى ذلك الحمض في سيريوم الدم . مستوى الدهون الكلية في سيريوم الدم زاد معنويا مع انخفاض مستوى حمض الاسكوربيك في الغذاء ، ولكنه لم يتأثر نتيجة الاجهاد الحرارى . تبين أنه من المرجح أن يكون لحمض الاسكوربيك دورا هاما في نمو الثدييات . تعزيز الغذاء المنخفض في حمض الاسكوربيك بمقدار كبير منه أدى الى أن تعود المعدلات البيولوجية لحيوانات التجارب الى الحالة العادية .