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Indications for adaptation to differently high vitamin C supplies in guinea pigs
1. Development of ascorbic acid levels after altered dosing

E. Degkwitz und R.-H. Bödeker

Biochemisches Institut der Universität Gießen
Institut für Medizinische Informatik der Universität Gießen

Summary: Guinea pigs supplied with 5 mg/100 g vitamin in the food seem to be at the marginal substitution for a survival and show no ability to adapt the ascorbic acid metabolism to this low support.

Guinea pigs longlastingly (6–8 weeks) supplied with 20 mg/100 g or 680 mg/100 g vitamin C in the food show the typical symptoms of an evolving adaptation by an overshooting in the course of the ascorbic acid levels in several organs after a switch to an extremely different vitamin C supply.

Zusammenfassung: Eine Substitution mit 5 mg/100 g Vitamin C im Futter stellt für Meerschweinchen anscheinend die untere Grenze dar, um ein Überleben zu ermöglichen. Sie scheinen keine Möglichkeit zu haben, den Stoffwechsel der Ascorbinsäure an diese niedrige Substitution zu adaptieren.

Meerschweinchen, die über lange Zeit (6–8 Wochen) 20 mg/100 g oder 680 mg/100 g Vitamin C im Futter erhalten hatten, zeigen, nachdem der Vitamin-C-Gehalt im Futter drastisch verändert worden ist, bei den Ascorbinsäurespiegeln mehrerer Organe das für eine ablaufende Adaptation typische Über- bzw. Unterschwingen.

Schlüsselwörter: Meerschweinchen, Ascorbinsäure, Adaption im Stoffwechsel; Vitamin C

Key words: guinea pigs, adaptation in ascorbic acid metabolism, blood, liver, spleen, adrenal glands

Introduction

There is a number of contradictious publications on the aspect of an adaptation in the metabolism of ascorbic acid to rather high vitamin C supply in guinea pigs (1, 8, 10). According to the achievements in biochemistry of that time there was neither a view of the possibility of a gradual adaptation of ascorbic acid metabolism corresponding to increasing vitamin C supply nor a view of the possibility of an adaptation mainly in specific organs and no imagination of an adaptation which exceeds ascorbic acid metabolism drawing into the general metabolic equilibrium. In this series (2 + 3) and in another (4 + 5) we will present indications that all three of these potentialities take place. Ascorbic acid levels as well as the amounts of hepatic and extrahepatic microsomal cytochromes and of hepatic mitochondrial cytochromes after altered dosing of vitamin C may show transient values during a period of about six-eight weeks before they adjust to a new adapted steady state. In ref. 4 and subsequent papers we describe unlike concentrations of diverse body substances in guinea pigs adapted to differently high vitamin C supplies.

In this paper we present investigations on the development of ascorbic acid levels in blood, liver, spleen and adrenals of guinea pigs which were at first longlastingly (6–8 weeks) accustomed to a defined supply with vitamin C and thereafter exposed to an extremely different level of substitution. There is agreement that in the case of adapting proceedings the development of the levels should show an overshoot as well after a decrease of the substitution in comparison with the initial supply as after an increase.

As examples for a decrease we investigated comparably the development of the ascorbic acid levels of two guinea pig groups. Both were at first accustomed to the very high supply with 680 mg/100 g vitamin C in the food. The one group was exposed to 5 mg/100 g thereafter, the other to 20 mg/100 g. As examples for an increase we as well investigated comparably two guinea pig groups. Both were at first accustomed to the rather low supply with 20 mg/100 g vitamin C in the food. The one group was exposed to 90 mg/100 g thereafter, the other to 680 mg/100 g.

Materials and Methods

The experiments were carried out in short- and smooth-haired yellow guinea pigs of own rearing. All animals were kept at 20 °C and at about 60 % humidity of the atmosphere in air conditioned rooms and were ad libitum provided with water. The guinea pigs breeding and their youngsters were kept in large cages and fed ad libitum the standard diet C 3012 from "Altromin" and supplementary hay and grass (summertime) or pieces of apples, powdered with vitamin C (wintertime).

The experimental guinea pigs had an initial body weight of about 400 g (i.e. an age of about six weeks) and were kept in makrolon cages M 4 with insert-floors out of stainless steel. They were accustomed to the semisynthetic diet C 3015 (without vitamin C) from "Altromin" for two weeks by a supply with a mixture (1:1) of the standard diet C 3011 and of the semisynthetic diet which was adjusted to 20 mg/100 g or 680 mg/100 g vitamin C in the food respectively. Thereafter the animals were fed with the sole supplemented semisynthetic diet for six – eight weeks, in order to bring out a possible adaptation to this supply. After this time the vitamin C content of the semisynthetic diet was altered as indicated. All guinea pigs investigated had gained body weight continuously. The animals were killed after one, two, four, six, eight or ten weeks after the supply with vitamin C had been changed. Therefore we obtained the values from every guinea pig only at one single point of time: ascorbic acid levels in four compartments, the amounts of hepatic microsomal protein of cytochrome P-450 and cytochrome b_5 (results published in 2) and the amounts of stored iron in the liver and in the spleen (results published in 3). The organs were dissected immediately and all materials were iced.

Ascorbic acid was determined by the method of Roe (9). It thereby was taken into account that the standard curves mostly do not cross the zero point.

Statistical analysis

The data observed were evaluated in the sense of exploratory data analysis under the assumption that the observed variables were normally or lognormally distributed. In order to test the influence of the factors [vitamin supply] or [time after altered dosing] two-factorial analysis of variance was used. If the H_0-hypothesis was rejected, groups of interest were compared by Scheffe's procedure.

Results

The values the guinea pig groups had started with before their supply with vitamin C was changed are not included in the following tables 1–8 and the corresponding statistical evaluations. We already knew that the ascorbic acid levels of the liver and the adrenal glands decline quickly and considerably after withdrawal of the vitamin (6) and that there is a corresponding recovery in the liver levels after renewed supply (7). Therefore that we could go in search of a possible adapting process already one week after a change in the substitution with vitamin C. The starting values are recorded in the legends of the corresponding tables.

a) Development of ascorbic acid levels after a decrease in supply from 680 mg/100 g to 20 mg/100 g and 5 mg/100 g vitamin C in the food respectively.

Table 1. Ascorbic acid levels in the blood. The time courses of the ascorbic acid levels in the blood in the two serial guinea pig groups show no parallelism within the time investigated ($p < 0.0001$). The levels declined after the decrease in the supply in both groups distinctly during the first week and to an equally low range. In the guinea pigs decreased to 5 mg/100 g the levels do not tend to further modifications thereafter. But they tend to an increase in the group decreased to 20 mg/100 g vitamin C in the food. The additional Scheffe-test yields $p < 0.0001$ between six and eight weeks.

Table 1. Ascorbic acid levels in the blood of guinea pigs reduced to 5 mg/100 g and 20 mg/100 g vitamin C in the food respectively from 680 mg/100 g (= starting value, mean: 1210 ± 140 [μg ascorbic acid/100 ml blood], n = 6).

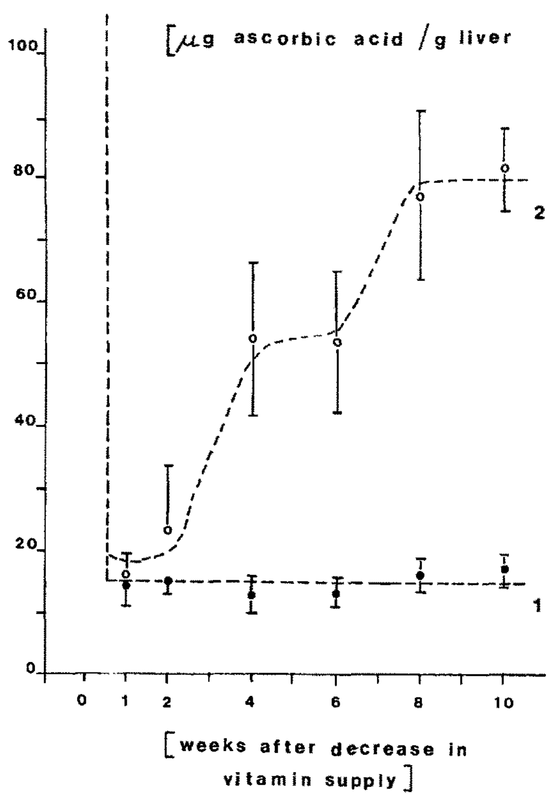
Combination A	B	n	[μg ascorbic acid/100 ml blood] mean	standard deviation
5	1	6	162.7	34.37
5	2	6	213.0	10.24
5	4	6	191.3	25.97
5	6	6	196.8	15.84
5	8	6	199.3	35.15
5	10	6	190.5	21.12
20	1	6	158.0	29.78
20	2	6	181.0	16.63
20	4	6	218.5	60.00
20	6	6	193.5	19.95
20	8	6	318.0	25.29
20	10	6	309.8	39.87

Combination:
A = [mg vitamin C/100 g food]
B = [weeks] after reduction of the vitamin supply

Table 2. Ascorbic acid levels in the liver of guinea pigs reduced to 5 mg/100 g and 20 mg/100 g vitamin C in the food respectively from 680 mg/100 g (= starting value, mean: 434 ± 34 [µg ascorbic acid/g liver], n = 6).

Combination		n	[µg ascorbic acid/g liver]	
A	B		mean	standard deviation
5	1	6	14.03	3.249
5	2	6	14.45	1.268
5	4	6	12.38	2.577
5	6	6	13.00	2.399
5	8	6	16.22	2.249
5	10	6	17.07	2.261
20	1	6	15.63	2.505
20	2	6	23.17	10.020
20	4	6	54.17	12.350
20	6	6	53.17	11.530
20	8	6	76.67	13.080
20	10	6	81.50	6.317

Combination:
A = [mg vitamin C/100 g food]
B = [weeks] after reduction of the vitamin supply



Ascorbic acid levels in the liver of guinea pigs decreased from [680 mg vitamin C/100 g food] to 5 mg/100 g (= 1) and to 20 mg/100 g (= 2).

Table 2. Ascorbic acid levels in the liver. The time courses of the ascorbic acid levels in the liver in the two serial guinea pig groups show no parallelism within the time investigated ($p < 0.0001$). The levels declined after the decrease in the supply in both groups dramatically during the first week and to an equally low range. In the guinea pigs decreased to 5 mg/100 g the levels do not tend to further modifications

Table 3. Ascorbic acid levels in the spleen of guinea pigs reduced to 5 mg/100 g and 20 mg/100 g vitamin C in the food respectively from 680 mg/100 g (= starting value, mean: 510 ± 18 [µg ascorbic acid/g spleen], n = 6).

Combination A	B	n	[µg ascorbic acid/g spleen] mean	standard deviation
5	1	6	66.42	9.178
5	2	6	55.53	12.280
5	4	6	57.35	11.870
5	6	6	58.93	6.041
5	8	6	66.17	7.404
5	10	6	59.77	6.519
20	1	6	92.33	23.050
20	2	6	95.18	28.010
20	4	6	177.00	32.980
20	6	6	147.70	30.240
20	8	6	230.80	21.530
20	10	6	225.30	20.800

Combination:
A = [mg vitamin C/100 g food]
B = [weeks] after reduction of the vitamin supply

Table 4. Ascorbic acid levels in the adrenal glands of guinea pigs reduced to 5 mg/100 g and 20 mg/100 g vitamin C in the food respectively from 680 mg/100 g (= starting value, mean: 1825 ± 130 [µg ascorbic acid/g adrenal], n = 6).

Combination A	B	n	[µg ascorbic acid/g adrenal gland] mean	standard deviation
5	1	6	183.3	21.72
5	2	6	200.2	11.82
5	4	6	184.3	11.04
5	6	6	183.0	12.10
5	8	6	197.0	7.58
5	10	6	188.2	17.10
20	1	6	242.2	43.44
20	2	6	240.5	37.73
20	4	6	568.0	56.97
20	6	6	407.3	62.76
20	8	6	457.0	43.06
20	10	6	445.3	38.75

Combination:
A = [mg vitamin C/100 g food]
B = [weeks] after reduction of the vitamin supply

thereafter. But in the group decreased to 20 mg/100 g vitamin C in the food they tend to a relatively high increase, therewith showing a deep overshoot (see fig.). The additional Scheffe-test yields $p < 0.0001$ between one and eight weeks.

Table 3. Ascorbic acid levels in the spleen. Table 4. Ascorbic acid levels in the adrenal glands. The time courses of the ascorbic acid levels both in the spleen and in the adrenal glands in the two serial guinea pig groups show no parallelism within the time investigated ($p < 0.0001$). The levels declined after the decrease in the supply distinctly in both groups during the first week and to an about similar low range. In the guinea pigs decreased to 5 mg/100 g the levels do not tend to further modifications thereafter. But they tend to increase in the group reduced to 20 mg/100 g vitamin C in the food. The rises may start later than in the corresponding levels in blood and liver. The additional Scheffe-tests yield $p < 0.0001$ between one and eight weeks as well for the ascorbic acid levels in spleen as in adrenal glands.

b) Development of ascorbic acid levels after an increase in supply from 20 mg/100 g to 90 mg/100 g and 680 mg/100 g vitamin C in the food respectively.

Table 5. Ascorbic acid levels in the blood. The time courses of the ascorbic acid levels in the blood in the two serial guinea pig groups show no parallelism within the time investigated ($p = 0.006$). The levels rose distinctly after the increase in the supply in both groups during the first week. In the guinea pigs raised to 90 mg/100 g the levels do not tend to further modifications thereafter. But the levels point to an overshoot in the first weeks in the group raised to 680 mg/100 g vitamin C in the food. The additional Scheffe-test yields $p = 0.001$ between one and eight weeks.

Table 5. Ascorbic acid levels in the blood of guinea pigs increased to 90 mg/100 g and 680 mg/100 g vitamin C in the food respectively from 20 mg/100 g (= starting value, mean: 310 ± 22 [µg ascorbic acid/100 ml blood] n = 6)

Combination A	B	n	[µg ascorbic acid/100 ml blood] mean	standard deviation
90	1	5	852.6	171.2
90	2	5	802.0	63.1
90	4	5	813.4	120.2
90	6	5	708.0	34.1
90	8	5	782.8	62.5
90	10	5	786.8	55.2
680	1	5	1422.0	129.2
680	2	5	1178.0	176.7
680	4	5	1344.0	161.9
680	6	5	1339.0	126.6
680	8	5	1046.0	123.2
680	10	5	1120.0	98.4

Combination:
A = [mg vitamin C/100 g food]
B = [weeks] after increase of the vitamin supply

Table 6. Ascorbic acid levels in the liver of guinea pigs increased to 90 mg/100 g and 680 mg/100 g vitamin C in the food respectively from 20 mg/100 g (= starting value, mean: 77 ± 12 [µg ascorbic acid/g liver] n = 6)

Combination A	B	n	[µg ascorbic acid/g liver] mean	standard deviation
90	1	5	251.0	36.18
90	2	5	279.2	24.08
90	4	5	268.4	30.99
90	6	5	250.0	25.42
90	8	5	259.8	29.13
90	10	5	255.0	13.42
680	1	5	403.6	49.92
680	2	5	406.0	29.73
680	4	5	450.4	58.59
680	6	5	424.6	41.31
680	8	5	439.2	40.93
680	10	5	412.6	48.98

Combination:
A = [mg vitamin C/100 g food]
B = [weeks] after increase of the vitamin supply

Table 6. Ascorbic acid levels in the liver. The time courses of the ascorbic acid levels in the two serial guinea pig groups in the liver show no tendency to interaction ($p > 0.6$) within the time investigated. But the levels are at different ranges ($p < 0.0001$). They are lower in the guinea pigs increased to 90 mg/100 g supply.

Table 7. Ascorbic acid levels in the spleen of guinea pigs increased to 90 mg/100 g and 680 mg/100 g vitamin C in the food respectively from 20 mg/100 g (= starting value, mean: 230 ± 20 [µg ascorbic acid/g spleen] n = 6)

Combination A	B	n	[µg ascorbic acid/g spleen] mean	standard deviation
90	1	5	493.4	30.00
90	2	5	504.6	16.68
90	4	5	470.2	20.71
90	6	5	483.6	24.76
90	8	5	463.2	30.49
90	10	5	462.8	46.75
680	1	5	515.0	23.05
680	2	5	598.6	43.43
680	4	5	539.0	27.54
680	6	5	552.8	30.95
680	8	5	506.2	24.26
680	10	5	508.0	31.50

Combination:
A = [mg vitamin C/100 g food]
B = [weeks] after increase of the vitamin supply

Table 7. Ascorbic acid levels in the spleen. The time courses of the ascorbic acid levels in the spleen in the two serial guinea pig groups do not tend to interactions ($p = 0.14$) within the time investigated. The levels are at different ranges ($p < 0.0001$). They are lower in the guinea pigs increased to 90 mg/100 g supply. Moreover the levels are in both groups unequal in the course of time ($p = 0.0001$). There is a slight overshoot.

Table 8. Ascorbic acid levels in the adrenal glands. The time courses of the ascorbic levels in the adrenal glands in the two serial guinea pig groups show no parallelism within the time investigated ($p < 0.0001$). In the guinea pigs raised to 90 mg/100 g the levels tend to an overshoot in the first three weeks. In the group raised to 680 mg/100 g they tend to a further rise during the middle of the adapting period. The additional Scheffe-test yields $p < 0.001$ between one and eight weeks for the 90 mg % guinea pigs and $p = 0.005$ between six and ten weeks for the guinea pigs with 680 mg/100 g supply.

Discussion

It is noticeable that the extent of the decrease of the ascorbic acid levels after a decrease in the supply from 680 mg/100 g to 5 mg/100 g or 20 mg/100 g vitamin C in the food are very different in the organs investigated. This points to rather different turnover rates for ascorbic acid within the organism. The extremely high losses in the liver suggest that this organ has a key function in ascorbic acid metabolism. This seems plausible since the liver is the only organ that is equipped for biosynthesis in those mammalian species that do not suffer the specific mal mutation of the δ-gulonolacton-oxidase which leaves the species of man, guinea pig and a

Table 8. Ascorbic acid levels in the adrenal glands of guinea pigs increased to 90 mg/100 g and 680 mg/100 g vitamin C in the food respectively from 20 mg/100 g (= starting value, mean: 450 ± 24 [µg ascorbic acid/g adrenal gland] n = 6)

Combination A	B	n	[µg ascorbic acid/g adrenal gland] mean	standard deviation
90	1	5	1763	97.3
90	2	5	1724	122.1
90	4	5	1657	77.0
90	6	5	1487	107.2
90	8	5	1342	123.8
90	10	5	1414	156.3
680	1	5	1790	57.0
680	2	5	1790	77.1
680	4	5	1856	91.8
680	6	5	1948	150.5
680	8	5	1785	70.7
680	10	5	1650	110.5

Combination:
A = [mg vitamin C/100 g food]
B = [weeks] after increase of the vitamin supply

few further mammalian and avian species to the need of substitution with exogenous ascorbic acid: "vitamin C".

Since guinea pigs are rather delicately minded and frequently equipped with a kamikaze-like mentality leading to a sudden refuse of food for several days, it seems worth while to check the course of the body weight of the animals before starting to investigate. The very quick fall of the ascorbic acid levels after a decrease in the supply will render at least for the ascorbic acid levels in the liver unphysiological mean values and deviations with regard to the defined supply with vitamin C if some of the guinea pigs had refused food in the last days. Ascorbic acid levels in the liver are on the other side a good characterization of the actual vitamin C status of the guinea pigs investigated. The frequent habit not to bother about the actual vitamin C status of the experimental guinea pigs very often lessens the validity of the results. The long adapting time as well is a warning that it is not sufficient to render guinea pigs with unknown previous vitamin C supply only to a short period of time for accustoming to an experimental diet.

The guinea pigs at first adapted to very high vitamin C supply (680 mg/100 g in the food) and then reduced to 5 mg/100 g thereafter apparently have no sufficient possibility to adapt their ascorbic acid metabolism to this low support, since the ascorbic acid levels slope down and remain at the low range. This view is supported by the nonparallelism of the ascorbic acid courses between the two groups reduced to low vitamin C supplies and is confirmed by additional investigations (2, 4 and subsequent papers).

A supply with 5 mg/100 g vitamin C in the food apparently is marginal for survival. We found no longlasting survival supporting guinea pigs with still lower amounts in the food.

The guinea pigs at first adapted to very high vitamin C supply (680 mg/100 g in the food) and then reduced to 20 mg/100 g thereafter show by the overshoot in the course of their ascorbic acid levels a typical symptom of an evolving adaptation. As to the adaptation mechanism the results might be produced multifactually. The relatively high incline of the liver levels suggest a reduced degradation of the vitamin. The increase of ascorbic acid levels in the blood might in part be due to an improved absorption in the intestine.

Additional investigations (2–4 and subsequent papers) will give evidence that the evolving adaptation is not restricted to ascorbic acid metabolism.

The guinea pigs which were at first adapted to the low vitamin supply with 20 mg/100 g and then exposed to the higher amounts as well showed the tendency to an overshoot of the ascorbic acid levels in several organs which is understood as a typical sign for an evolving adaptation. These results complete the indications for an ability of guinea pigs to adapt their ascorbic acid metabolism to the extent of the vitamin C supply. The lack of an adaptation symptom in the liver might be incidental, due to a playing together of an increasing degradation of ascorbic acid in the liver and a reduction of the elimination of ascorbic acid in the urin. For we found a reduced elimination of ascorbic acid in the urin in guinea pigs adapted to high vitamin supply (5).

The ability to adaptation of the ascorbic acid metabolism in guinea pigs seems not too great a surprise after particular consideration. The guinea pig has a mal mutation, suffering the defectiveness of one single enzyme. There is no reason to expect that this may impair the key functions for the regulation of the ascorbic acid metabolism. All the other species, equipped for biosynthesis of ascorbic acid as well take up exogenous ascorbic acid with the food and hence ought to be disposed to protect their organism in the case of an excess from the exogenous sources by adaptation of its metabolism. In this plausible case guinea pigs would but make use of the traditional mammalian protection.

The very long adaptating period probably explains why many investigations were not successful, most investigators did not examine a longer course of time than two-three weeks after a switch of the vitamin C supply.

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Authors' address:

Prof. Dr. E. Degkwitz, Biochemisches Institut, Friedrichstraße 24, D-6300 Gießen, Federal Republic of Germany