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Indications for adaptation to differently high vitamin C supplies in guinea pigs
2. Development of hepatic amounts of microsomal protein and cytochromes (P-450, b_5) after altered dosing

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Summary: Guinea pigs longlastingly (6–8 weeks) supplied with 680 mg/100 g vitamin in the food and for ten weeks thereafter exposed to:
5 mg/100 g: show no ability to maintain the amounts of hepatic microsomal cytochrome P-450
20 mg/100 g: show the typical symptom of an evolving adaptation by an overshoot of the amounts of hepatic microsomal cytochrome P-450.

Zusammenfassung: Meerschweinchen, die über lange Zeit (6–8 Wochen) 680 mg/100 g Vitamin C im Futter erhalten hatten und dann 10 Wochen lang substituiert wurden mit
5 mg/100 g, konnten in der Leber nicht die Gehalte an mikrosomalem Cytochrom P-450 aufrechterhalten
20 mg/100 g, zeigten in der Leber das für eine Adaptation typische Unterschwingen bei dem Gehalt an mikrosomalem Cytochrom P-450.

Key words: guinea pigs, adaptation of hepatic microsomal proteins and cytochromes P-450 and b_5 to different vitamin C supplies

Schlüsselwörter: Meerschweinchen, mikrosomales Protein; Cytochrome P-450 und b_5; Vitamin-C-Substitution

Introduction

In preceding investigations (3, 4, 8, 9) we found a decrease of the hepatic microsomal protein and cytochromes P-450 and b_5 in guinea pigs completely unsupplied with vitamin C. Further research on the cytochromes pointed to alterations in the metabolism of the heme part (8 and 9). But in our opinion the frequently utilized model system of total lack of vitamin C supply turned out unsatisfacory. The guinea pigs die after about four weeks and the metabolic equilibrium is progressively deranged in favour of the catabolic reactions without any prospect of a new steady state, enabling far-reaching investigations. It seemed more favourable to examine if the microsomal protein and cytochrome decreases are restricted to complete lack of vitamin C or if they occur as well after a reduction of the supply.

The experimental guinea pigs were the same as in number one of this series (5). But we renounced estimations already after one week after the change of the vitamin C supply and also a guinea pig group with the increase to 90 mg/100 g supply after adaptation to 20 mg/100 g vitamin C in the food.

Materials and Methods

For the guinea pigs see ref. 5, with the exception that no animals were already killed after one week.

For the separation of liver microsomes and the determination of the cytochromes P-450 and b_5 see ref. 3.

Protein was determined by the method of Beisenherz et al. (1) but turbidities were estimated by the method of Bode et al. (2) additionally.

Statistical analysis

For the serial guinea pig groups decreased to 20 mg/100 g and 5 mg/100 g vitamin C in the food see ref. 5.

For the serial guinea pig groups raised to 680 mg/100 g see ref. 7. H_0: there is no influence of the time after altered dosing on the amounts of hepatic microsomal protein and cytochromes.

Results

The values the guinea pig groups had started with before their supply with vitamin C was changed were included in the following tables 1–6 (= zero weeks) and in the corresponding statistical evaluations. Since we

Table 1. Amounts of hepatic microsomal protein in guina 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 = zero weeks).

Combination A	Combination B	n	[mg protein/g liver] mean	standard deviation
5	0	6	21.57	1.611
5	2	6	16.67	2.165
5	4	6	16.68	2.715
5	6	6	16.42	2.740
5	8	6	20.27	2.329
5	10	6	20.88	3.555
20	0	6	21.57	1.611
20	2	6	20.70	1.990
20	4	6	17.58	2.549
20	6	6	18.83	2.270
20	8	6	18.95	3.140
20	10	6	23.30	1.390

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

Table 2. Amounts of hepatic microsomal cytochrome P-450 in 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 = zero weeks).

Combination A	B	n	[n mol cyt. P-450/mg protein] mean	standard deviation
5	0	6	1.045	0.084
5	2	6	0.851	0.084
5	4	6	0.776	0.150
5	6	6	0.713	0.064
5	8	6	0.613	0.060
5	10	6	0.676	0.064
20	0	6	1.045	0.084
20	2	6	0.851	0.039
20	4	6	0.828	0.031
20	6	6	1.057	0.096
20	8	6	0.945	0.049
20	10	6	0.935	0.044

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

already knew (3) that the turnover times of the microsomal protein and the two cytochromes are far longer than those of the ascorbic acid levels investigated in ref. 5 and that the margin within the values are modified are rather narrow it seemed adequate to include the starting values (in opposition to ref. 5).

Table 3. Amounts of hepatic microsomal cytochrome b_5 in 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 = zero weeks).

Combination A	B	n	[n mol cyt. b_5/mg protein] mean	standard deviation
5	0	6	0.760	0.081
5	2	6	0.743	0.055
5	4	6	0.786	0.053
5	6	6	0.643	0.038
5	8	6	0.616	0.099
5	10	6	0.623	0.101
20	0	6	0.760	0.081
20	2	6	0.685	0.060
20	4	6	0.770	0.053
20	6	6	0.881	0.123
20	8	6	0.878	0.097
20	10	6	0.823	0.041

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

Table 4. Amounts of hepatic microsomal protein in guinea pigs increased to 680 mg/100 g vitamin C in the food from 20 mg/100 g (= starting value = zero weeks).

[weeks]*	n	[mg protein/g liver] mean	standard deviation
0	6	18.89	2.205
2	6	19.44	1.744
4	6	18.20	2.511
6	6	21.37	2.254
8	6	21.04	2.254
10	6	21.32	1.701

*[weeks] after increase of the vitamin supply

a) Development of the amounts of hepatic microsomal protein and cytochromes P-450 and b_5 after change of the vitamin supply from 680 mg/100 g to 20 mg/100 g and 5 mg/100 g vitamin C in the food respectively.

Table 1. Amounts of hepatic microsomal protein. The time courses of the amounts of hepatic microsomal protein in the two serial guinea pig groups show no indication of interaction after the decrease in the supply ($p > 0.1$). The amounts are unequal in the course of time ($p < 0.001$), showing a slight tendency to a transient decrease and a recovery after at latest ten weeks.

Table 2. Amounts of hepatic microsomal cytochrome P-450. The time courses of the amounts of hepatic microsomal cytochrome P-450 in the two serial guinea pig groups show no parallelism after the decrease in the vitamin supply ($p < 0.001$). In the guinea pigs exposed to 5 mg/100 g supply the amounts decline progressively and do not recover. The additional Scheffe-test yields $p < 0.001$ for the decrease after eight and ten weeks compared with the initial amount. In the guinea pigs exposed to 20 mg/100 g supply the amounts decrease within the first four weeks and recover thereafter. The additional Scheffe-test yields $p < 0.001$ as well for the decrease after four weeks compared with the initial amount as for the increase between four and six weeks.

Table 5. Amounts of hepatic microsomal cytochrome P-450 in guinea pigs increased to 680 mg/100 g vitamin C in the food from 20 mg/100 g (= starting value = zero weeks).

[weeks]*	n	[n mol cyt. P-450/mg protein] mean	standard deviation
0	6	1.001	0.093
2	6	1.141	0.078
4	6	1.088	0.141
6	6	1.040	0.077
8	6	1.020	0.111
10	6	1.031	0.165

*[weeks] after increase of the vitamin supply

Table 6. Amounts of hepatic microsomal cytochrome b_5 in guinea pigs increased to 680 mg/100 g vitamin C in the food from 20 mg/100 g (= starting value = zero weeks).

[weeks]*	n	[n mol cyt. P-450/mg Protein] mean	standard deviation
0	6	0.825	0.095
2	6	0.711	0.114
4	6	0.685	0.089
6	6	0.738	0.104
8	6	0.760	0.060
10	6	0.808	0.117

*[weeks] after increase of the vitamin supply

Table 3. Amounts of hepatic microsomal cytochrome b_5.

The time courses of the amounts of hepatic microsomal cytochrome b_5 in the two serial guinea pig groups show no parallelism after the decrease in the vitamin supply ($p < 0.0001$). In the guinea pigs exposed to 5 mg/100 g supply the amounts tend to a decline. The additional Scheffe-test for the difference in the amounts between the groups exposed to 5 mg/100 g and 20 mg/100 g vitamin at ten weeks C yields $p < 0.001$. In the guinea pigs exposed to 20 mg/100 g supply the amounts tend to a transient decrease and a recovery after six weeks. The additional Scheffe-test yields $p < 0.01$ for the increase between two and six or ten weeks.

b) Course of amounts of hepatic microsomal protein and cytochromes P-450 and b_5 after change of the vitamin supply from 20 mg/100 g to 680 mg/100 g vitamin C in the food.

Table 4. Amounts of hepatic microsomal protein.

The amounts are not equal in the time investigated ($p = 0.04$) but do not show an evident overshoot.

Table 5. Amounts of hepatic microsomal cytochrome P-450.

The amounts tend to no modifications in the time investigated ($p > 0.4$).

Table 6. Amounts of hepatic microsomal cytochrome b_5.

The amounts tend to no modifications in the time investigated ($p > 0.3$).

Discussion

The results in the guinea pigs reduced to 5 mg/100 g vitamin C in the food are analogous to the outcome in the ascorbic levels (5) with exception of a rather slight overshoot in the microsomal protein. These results confirm the impression that such guinea pigs lost sufficient possibilities to adapt to this low support. Further investigations (6) demonstrated that the decrease of cytochrome P-450, which is known to play a role in the metabolism of exogenous and endogenous water-unsoluble substances, causes elongated sleeping times after application of evipane.

The finding that 5 mg/100 g in the food is an about marginal supply for survival (see also ref. 5) is confirmed by the outcome that the marginal support is dependent on the manner of the supply. The amounts of the hepatic microsomal cytochrome P-450 are not decreased, if the guinea pigs receive the summarized lot of vitamin C that they would consume in the

course of one week in two single portions applicated via stomach tube at an interval of three and a half days (6).

The guinea pigs reduced to 20 mg/100 g vitamin C in the food show an evident overshoot in the amounts of hepatic microsomal cytochrome P-450 and hence a typical symptom of an evolving adaptation. In the amounts of the hepatic microsomal protein and cytochrome b_5 there is but a faint overshoot. Since the margins within the values are modified are rather narrow it is difficult to perceive a clear overshoot of these amounts. The results are in part conform with our findings in the model system of total lack of vitamin C supply. For we as well found a larger and faster decline of the hepatic microsomal cytochrome P-450 than of the microsomal protein and cytochrome b_5 under these conditions (3) apparently due to longer half-life times of the latter. It seems sensible that it is easier to perceive an overshoot of a substance with a shorter half life and wider margins within the values are modified like cytochrome P-450. Hence an overshoot of the amounts of microsomal protein and cytochrome b_5 may come into question as well.

Since guinea pigs adapted to 20 mg/100 g vitamin C in the food have the same amounts of hepatic microsomal cytochromes P-450 and b_5 like such adapted to 680 mg/100 g an overshooting might only be expected in guinea pigs at first adapted to 5 mg/100 g supply and exposed to higher levels thereafter. But according to the small differences between the amounts and the limited sensibility of the determination of the cytochromes we renounced an investigation of this kind.

As to the mechanism of the decreases in the amounts of the hepatic microsomal cytochromes previous investigations (8) especially on cytochrome P-450 in the model system of complete lack of vitamin C supply already had shown, that the low amounts are only indirectly due to the suboptimal ascorbic acid levels, since the amounts could be raised by other substances than ascorbic acid, for example by δ-aminolaevulinic acid or by phenobarbital. If it is taken into account that the two substances stimulate quite different metabolic pathways more over that the adapting needs the long period of six-eight weeks and that long lasting adapting times usually include an involvement of slowly acting hormones it could be taken into consideration that the remaining low amounts of cytochrome P-450 (and b_5) and the remaining low levels of ascorbic acid in the guinea pigs adapted to 5 mg/100 g vitamin C in the food are the result of a certain hormonal constellation reigning the metabolism. For that reason we compared common clinical biochemical parameters in guinea pig groups adapted to a defined vitamin C supply in a suit between 5 mg/100 g and 680 mg/100 g in the food (7).

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