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Activity of alkaline phosphatase in the serum of normal and of ascorbic-acid-deficient guinea pigs

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Introduction

The activity of the alkaline phosphatase in the serum is thought to be generally decreased in guinea pigs deprived of ascorbic acid (1). This concept is based on research in guinea pigs completely deprived of vitamin C for 2 weeks (2–6), i.e., in an acute deficiency system. But Ginter et al. (7) and we as well did not find decreased enzyme levels in guinea pigs slowly reduced in vitamin C by restriction in ascorbic-acid supply. This is a contradiction. If ascorbic-acid deficiency is essentially connected with a decrease of the activity of the alkaline phosphatase in the serum, this symptom should be independent from the procedure inducing lack of ascorbic acid.

With regard to this contradiction we carried out the following investigations:

1. We studied the relationship between the activity of alkaline phosphatase in the serum and the age in guinea pigs. It is well known that the alkaline phosphatase mainly originates from the bones and that its serum levels are a direct reflection of the osteoblastic activity and hence of growth intensity. Accordingly, serum enzyme levels decrease physiologically with increasing age. In this respect, enzyme levels of the ascorbic-acid-deficient guinea pigs were compared with those in normal animals of the same age.
2. We induced equally low ascorbic-acid levels in guinea pigs by two different procedures: a) complete deprivation of the vitamin for 2 weeks; b) restriction to 50 mg vitamin C/kg diet for 6–8 weeks. A comparison of the serum enzyme levels in guinea pigs made deficient in ascorbic acid by different procedures seems necessary.
3. We estimated the serum activity of the alkaline phosphatase in guinea pigs well supplied with ascorbic acid but restricted in food for two weeks, since a previous paper (5) reported a decrease of the enzyme level in guinea pigs limited in food and not supplemented with ascorbic acid.

Methods

Estimation of alkaline phosphatase

The enzyme activity was estimated by the optimized standard method of the "Deutsche Gesellschaft für Klinische Chemie" (8, 9). The activity is expressed in U/l (25 °C).

Blood specimens were collected from the guinea pigs between 8 and 9 hrs. a.m. The animals were not fasted in order to avoid alterations in the endocrinological regimen.

Estimation of ascorbic acid

Ascorbic acid was determined by the method of Roe as "total ascorbic acid" (10).

Guinea pigs

a) Supply with ascorbic acid

Guinea pigs well supplied with vitamin C were fed ad libitum with standard diet VM 3012 from Altromin, supplemented by hay and grass. In order to obtain high ascorbic-acid levels, the guinea pigs restricted in food were fed by stomach tube 3 times per day with 200 mg Na-ascorbate per kg body weight.

Guinea pigs were made deficient in ascorbic acid by feeding ad libitum the semisynthetic diet C 30353 from Altromin. The one group received the diet free of ascorbic acid, the other with 50 mg vitamin C/kg diet. Both groups were adapted to the semisynthetic diet by two steps. First step: Supply with a mixture of the standard and the semisynthetic diet (1:1) completed to 3.5 g vitamin C/kg diet for two weeks. Second step: supply with the semisynthetic diet completed to 6.7 g vitamin C/kg diet for 2-3 weeks.

b) Ascorbic-acid levels

Guinea pigs well supplied with ascorbic acid showed levels of 300 ± 30 mg ascorbic acid/kg liver fresh weight.

Guinea pigs deficient in ascorbic acid showed levels of 20 ± 10 mg ascorbic acid/kg liver fresh weight.

c) Increases in body weight

Guinea pigs well supplied with ascorbic acid and fed ad libitum gained weight continuously until the time of killing. Animals restricted in food intake for 2 weeks lost $15 \pm 2\%$ of body weight in this time.

Guinea pigs completely deprived of vitamin C for 2 weeks either maintained constant body weight or lost $8 \pm 2\%$ in this time.

Most guinea pigs restricted in ascorbic acid stopped weight increase for about one week during the adaptation to the diet, the four smallest animals for about four weeks.

Results

The declining straight line in the figure is a fitted curve presenting the relation between the activity of the alkaline phosphatase in the serum and the body weight of untreated guinea pigs well supplied with ascorbic acid. The body weight was used as the index of age and hence of growth intensity. The hatched area represents the standard deviation.

All the marks in the figure are data of individual guinea pigs treated as indicated. Since body weight is no more a valid index of age in animals which suffered a fall in weight, the data of such guinea pigs had to be adjusted and hence are especially marked. The serum-enzyme activity is correlated with the maximum weight of the animals during experimentation.

Guinea pigs restricted in vitamin C

Most of the enzyme levels were within the deviation of the levels in untreated animals. Three animals had higher levels than normal.

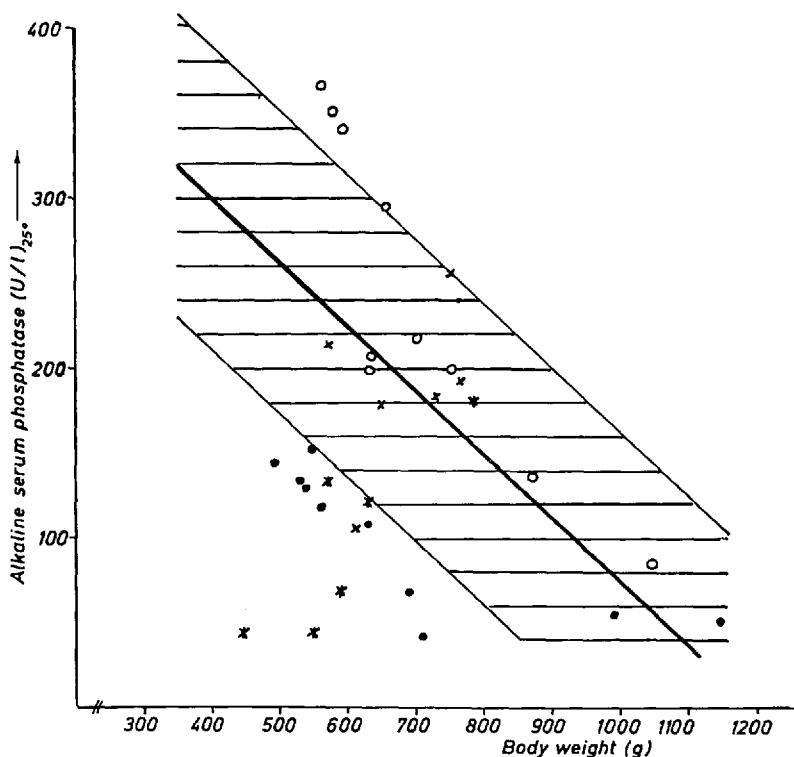


Fig. 1. Activity of alkaline phosphatase in the serum of guinea pigs with reference to their body weights. The data of 45 normal animals with body weights ranging from 325 to 1175 g (uniformly distributed in this range) are presented by a fitted curve

$$y = 446.65 - 0.36 x - 5.90 x^2$$

correlation coefficient = 0.90

The hatched area represents the $2 S_{y \cdot x}$ deviation of the y-axis (88.2 U/l).

The data of the following animals are marked individually:

- Guinea pigs restricted to 50 mg vitamin C/kg diet for 6–8 weeks (number of animals: 10)
- × Guinea pigs completely deprived of vitamin C for 2 weeks but with constant body weights (number of animals: 6)
- * Guinea pigs completely deprived of vitamin C for 2 weeks but with falls in body weights (number of animals: 6)
- Guinea pigs restricted in food intake (number of animals: 10) and consequently with falls in body weights.

Enzyme levels of animals which lost weight during experimentation were adjusted (see text).

Guinea pigs completely deprived of vitamin C

Some animals showed enzyme activities within the limits of the untreated group, some considerably lower values. But all except one of the animals with decreased serum alkaline phosphatase levels had suffered a fall in body weight.

Guinea pigs well supplied with ascorbic acid but restricted in food

All except two rather old animals showed enzyme activities below the standard deviation of untreated animals.

Discussion

Because of the steep linear relationship between the alkaline phosphatase activity in the serum and growth intensity in guinea pigs, the habit of comparing enzyme levels in animals of different ages is inappropriate. The steep incline may afford an adjustment of the data of treated guinea pigs. E.g. reference of the serum enzyme level to the body weight at the time of estimation will be misleading in guinea pigs which had suffered a fall in weight. The development preceding the experimental period may have to be taken into consideration as well. The increase of the enzyme level in the three smallest guinea pigs restricted in ascorbic acid may not reflect true conditions. These animals stopped weight increases for about four weeks during adaptation. Hence the correlation between body weight, age, and expectable growth intensity was altered. Reports on increased serum levels of alkaline phosphatase in guinea pigs restricted in ascorbic acid (7) probably result from such conditions. Other previous reports may be as well not always based on appropriate controls.

The data demonstrate that the serum alkaline phosphatase is not generally, i.e. essentially, decreased in guinea pigs with low ascorbic-acid levels. Declines of the enzyme levels seem to take place only in special circumstances. The mechanism causing the decline has not yet been investigated. Since the osteoblast activity is controlled by growth hormone, the effect of ascorbic-acid deficiency may be indirect, by the circumstance that the procedure of inducing ascorbic-acid deficiency coincidentally induces a metabolic state that inhibits the secretion of growth hormone. The results in guinea pigs well supplied with ascorbic acid but limited in food show that the enzyme level even may be decreased by falls in weight in spite of a high vitamin C status.

It has to be taken into consideration that the conditions inducing ascorbic-acid deficiency contribute to the disease course:

Guinea pigs completely deprived of vitamin C suffer the known scorbutic hemorrhages within 3 weeks (11). They lose weight and die about 1 week later. Their ascorbic-acid levels decline continuously during experimentation (12).

Guinea pigs restricted to 50 mg vitamin C/kg diet suffer scorbutic hemorrhages but not before about 20 weeks. They may not gain weight during the first week of experimentation but will do so continuously thereafter. They survive the restriction of the vitamin for more than 25 weeks (unpublished results). Their ascorbic-acid levels decline in the beginning but become constant after about 5-6 weeks. They adapt to low ascorbic-acid levels in this period of time (13).

The dissimilarity between the two disease courses suggests that the symptoms in guinea pigs completely deprived of vitamin C are multifactorial. Since this animal species is known to be very sensitive to changes in diet, it is tempting to speculate that stress reactions contribute to the

disease, which develops after complete omission of the vitamin, i.e. in acute deficiency, and that they may decrease serum levels of alkaline phosphatase. It is well known that stress syndrome is initiated by increased secretion of glucocorticoids. A decrease of somatomedine levels and consequently cessation of growth have been claimed to occur in status of high glucocorticoid levels (14).

Even the manner of application of vitamin C seems to play a role in minimal supply. Our guinea pigs did not survive a diet restricted to 20 mg ascorbic acid/kg diet (adequate to 0.5–0.6 mg ascorbic acid per day, dependent on body weight, unpublished results). But Ginter et al. (7) reported a survival of guinea pigs on a dosage of 0.5 mg per day, administered orally every other day.

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Summary

Guinea pigs well supplied with vitamin C show a linear relation between serum alkaline phosphatase activity and body weight. Investigations in guinea pigs induced to low ascorbic-acid levels by two different procedures (complete and partial deprivation of vitamin C) demonstrated that the activity of the alkaline phosphatase is not generally decreased in the serum of ascorbic-acid-deficient guinea pigs. Decreases of the serum enzyme levels reported in guinea pigs completely deprived of vitamin C, i.e. in acute deficiency, may be due to stress factors.

Zusammenfassung

Bei Meerschweinchen, die gut mit Vitamin C versorgt sind, findet sich eine lineare Beziehung zwischen der Aktivität der alkalischen Phosphatase im Serum und dem Körpergewicht. Untersuchungen bei Meerschweinchen, in denen durch zwei verschiedene Verfahren (völliger und partieller Entzug) niedrige Ascorbinsäurespiegel herbeigeführt worden waren, zeigten, daß die Aktivität der alkalischen Phosphatase im Serum nicht generell bei Vitamin-C-Mangel erniedrigt ist. Abnahmen der Enzymspiegel im Serum, die sich bei Meerschweinchen nach völligem Entzug von Vitamin C finden, d. h. bei akutem Mangel, sind vermutlich die Folge von Stressfaktoren.

Enzyme: Alkaline phosphatase, orthophosphoric monoester phosphohydrolase (EC 3.1.3.1.)

Key words: serum alkaline phosphatase, ascorbic-acid deficiency, guinea pigs

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