levels of 750 mg/kg was performed. Results confirmed the initial impression: the loss in enzyme activity in liver caused by DEDC was 52±13%, while that for DMDC was 86±5% (mean±SD, for 6 mice in each group; $p < 0.05$).

Discussion. DMDC, FLA-8, FLA-35 and FLA-57, as well as DEDC[2], can be used to reversibly inhibit the activity of the copper-zinc form of SOD in studies performed in vitro. Inhibited enzyme preparations can be dialyzed to provide inactive enzyme free of excess inhibitor. Reversal of inhibition can be achieved with $CuSO_4$. For in vivo studies, DMDC presents an alternate to the use of DEDC to inhibit tissue SOD.

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Serum triglycerides and post-heparin lipolytic activity in guinea-pigs with latent vitamin C deficiency

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Summary. Guinea-pigs with latent vitamin C deficiency have a raised serum triglyceride concentration and significantly reduced post-heparin lipolytic activity in blood plasma.

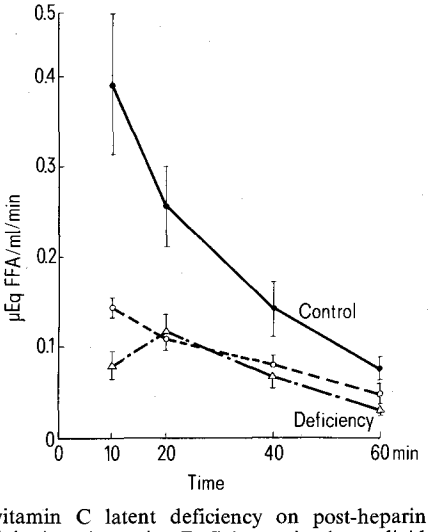
Microsomal 7α-hydroxylation of cholesterol, a rate-limiting reaction in the transformation of cholesterol to bile acids, slows down in the liver of guinea-pigs with latent ascorbic acid deficiency[1,2]. In consequence, cholesterol catabolism also slows down, hypercholesterolemia develops, cholesterol accumulates in certain tissues and, in long-term experiments, pathological changes may occur in the vascular system[3-5]. In recent years, evidence has accumulated showing that latent vitamin C deficiency also affects plasma triglyceride metabolism[6-10]. This study is a contribution to knowledge of the mechanism of the development of hypertriglyceridemia in guinea-pigs with chronic latent vitamin C deficiency.

Material and methods. We used growing male guinea-pigs fed ad libitum on a scorbutogenic diet[11] containing 15% w/w fat, the main source of which in this diet is butter and dried milk.

Experiment I. Guinea-pigs with an initial weight of 350 g were put on 3 very different doses of ascorbic acid. In the 1st group, latent vitamin C deficiency was induced by our routine method: for 2 weeks the animals were given a vitamin C-free diet and then received a maintaining dose of 0.5 mg ascorbic acid/animal/day. The 2nd group was given the same diet plus 0.05% w/w crystalline ascorbic acid and the 3rd group the same diet plus 0.5% w/w ascorbic acid. The last 2 diets were freshly prepared twice a week and were kept in the cold in sealed plastic vessels. 3 other groups of guinea-pigs had the same graded ascorbic acid intake, but their diet contained 0.2% w/w cholesterol dissolved in butter. The experiment lasted 17 weeks. The guinea-pigs were then deprived of food; 18 h later they were killed under mild ether anesthesia and the triglyceride (Boehringer UV test) and vitamin C concentration[12] were determined in their serum.

Experiment II. Guinea-pigs with an initial weight of 370 g were given the basic scorbutogenic diet without added cholesterol. In the 1st group, latent vitamin C deficiency was induced in the same way as in experiment I; for the 2nd group, 0.5% w/w ascorbic acid was added to the diet. The experiment lasted 9 weeks. The animals were then deprived of food for 20 h and the total lipolytic activity of post-heparin plasma was determined in blood samples taken from the jugular vein of thiopental-anesthetized animals 10, 20, 40 and 60 min after the i.v. administration of heparin (Spofa) in a dose of 5 U/100 g b. wt. Lipolytic activity was determined by quantification of the fatty acids[13] released during the incubation of 0.2 ml plasma in medium containing intralipid (Vitrum) as substrate[14].

Results and discussion. Experiment I. The weight curves in all the groups were similar, and at the end of the experiment the animals b. wt was 600-650 g. Table 1 shows that the serum vitamin C level rose together with the ascorbic acid intake, while the triglyceride concentration fell. There is a moderately close negative linear correlation between the serum vitamin C and triglyceride level ($r_{xy} = -0.548$, $p < 0.002$): in the presence of high vitamin C levels, the triglyceride level is low and vice versa. Several authors have described an incidence of hypertriglyceridemia in the presence of vitamin C deficiency[6,7,9]. The hypotriglyceridemic effect of large doses of ascorbic acid has also been described, in guinea-pigs[7,10,15], rabbits[16], golden hamsters[9], monkeys[17,18] and man[9,16]. It is interesting to note that the addition of cholesterol to the diet of guinea-pigs given small doses of ascorbic acid caused a



Effect of vitamin C latent deficiency on post-heparin plasma lipolytic activity in guinea-pigs. Deficient animals are divided into 2 subgroups: 1: Maximum activity was reached within the same period of time as in control, that is, 10 min after heparin administration; 2: maximum activity was reached even 20 min after heparin administration, ±SEM is given by verticals at each value.

Table 1. The effect of graded doses of ascorbic acid on the vitamin C and triglyceride levels in the blood serum of guinea-pigs fed basal or cholesterol diets

Diet	Parameter	Ascorbic acid intake 0.5 mg/animal/24 h	0.05% in diet	0.5% in diet
Basal	Vitamin C (mg/100 ml)	0.10 ± 0.01^a (9)	0.40 ± 0.03^b (12)	1.17 ± 0.12^c (12)
	Triglycerides (mg/100 ml)	$229 + 27^a$ (11)	168 ± 8^b (11)	99 ± 8^c (13)
Cholesterol	Vitamin C (mg/100 ml)	0.14 ± 0.02^a (12)	0.30 ± 0.02^b (11)	1.02 ± 0.03^c (12)
	Triglycerides (mg/100 ml)	138 ± 13^a (12)	139 ± 12^a (11)	98 ± 5^b (12)

Figures represent mean values ± SEM. The numbers in parenthesis denote the number of animals observed. Means not followed by the same letter superscript are significantly different ($p < 0.05$–0.001, t-test).

Table 2. The effect of marginal vitamin C deficiency on the postheparin lipolytic activity of plasma in guinea-pigs

Time after heparin administration (min)	Ascorbic acid intake 0.5 mg/animal/24 g	0.5% in diet	Statistical significance (t-test)
10	0.115 ± 0.013 (13)	0.391 ± 0.078 (10)	$p < 0.001$
20	0.114 ± 0.011 (13)	0.257 ± 0.044 (10)	$p < 0.002$
40	0.076 ± 0.008 (13)	0.144 ± 0.029 (10)	$p < 0.02$
60	0.041 ± 0.002 (12)	0.078 ± 0.013 (10)	$p < 0.02$

Figures represent mean values (μmoles free fatty acid/ml/min) ± SEM. The numbers in parenthesis denote the number of animals observed.

drop in the plasma triglyceride level. This phenomenon is probably associated with the pronounced effect of exogenous cholesterol on the composition of the plasma lipoproteins in guinea-pigs[19].

Experiment II. At the end of the experiment, b. wt in the 2 groups was practically the same (in the region of 440 g). The response of deficient animals to the injection of heparin was significantly weaker than that of the controls (table 2): 10 min after heparin administration lipolytic activity in the deficient group was only one-third of the control values and 60 min after it was still significantly lower. Moreover, in half of the deficient animals, the kinetics of lipolytic activity were also changed (figure), with a shift of maximum activity to the 20th min after the injection of heparin.

The total lipolytic activity of post-heparin plasma comprises the sum of 2 main lipolytic enzyme activities released from various tissues into the blood by the action of heparin: a) lipoprotein lipase (EC 3.1.1.3), the quantitatively most important source of which in guinea-pigs, as in other animals, is adipose tissue, skeletal muscle and the heart[20]; b) triglyceride lipase released from the liver. Liver triglyceride lipase and cardiac lipoprotein lipase are significantly elevated in guinea-pigs with latent vitamin C deficiency[7]. Large doses of vitamin C inhibit cardiac lipoprotein lipase in monkeys[21]. The low post-heparin lipolytic activity in the plasma of vitamin-deficient guinea-pigs is therefore probably due to low lipoprotein lipase activity in their adipose tissue and/or skeletal muscle. Preliminary results in guinea-pigs put on a graded ascorbate intake indicate that lipoprotein lipase activity in the adipose tissue of guinea-pigs with marginal vitamin C deficiency is lower than in guinea-pigs with a high ascorbate intake[22]. On the other hand, ascorbic acid inhibits hormone-sensitive lipase from adipose tissue in vitro[23]. It is therefore likely that vitamin C interferes, by a still unidentified mechanism, with the interplay of lipolytic reactions and in this way influences the serum triglyceride level.

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