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Plasma Lipids in Scurvy: Effect of Ascorbic Acid Supplement and Insulin Treatment.* (28049)

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Banerjee *et al.*(1) reported that total body cholesterol was increased in scorbutic guinea pigs, and that insulin treatment of the scorbutic animals reduced body cholesterol to normal. Body cholesterol was further increased when scorbutic guinea pigs were fed acetate(2). The clearance of fat from blood has been suggested to be dependent on normal carbohydrate utilization(3). The diminished glucose tolerance of scorbutic guinea pigs seemed to be due to the diminished insulin formation associated with the condition (4,5). Utilization of carbohydrate inhibits release of nonesterified fatty acids (NEFA) from fat depots(6). It was, therefore, of interest to estimate the different fractions of plasma lipids, *e.g.*, triglycerides, NEFA, cholesterol, phospholipids, lipoproteins and beta-lipoprotein cholesterol in scorbutic guinea pigs, and to study the effects of insulin treatment and ascorbic acid supplementation on these lipids.

Materials and methods. Male guinea pigs weighing about 300 g and maintained on a scorbutogenic diet(7), 5 mg ascorbic acid per day and 2 drops of a concentrate of Vit. A and D twice a week, were divided into groups of 3 animals, one normal, one scorbutic and one insulin-treated scorbutic. The amount of diet consumed per day by the scorbutic member of a group was fed to the other 2 members of the group on the subsequent day. The 'normal' animals were fed 5 mg ascorbic acid per animal per day. From the 10th day of the experiment the animals intended for insulin treatment were given a daily subcutaneous injection of protamine zinc insulin in a dose increasing from 0.1 unit per 100 g body weight to 0.3 unit throughout the experimental period. Another batch of guinea pigs was fed the scorbutogenic diet *ad libitum*. When symptoms of scurvy developed, they were fed a daily dose of 10 mg ascorbic acid for 4 weeks. By this time the animals had completely recovered from scurvy. In the fourth week of experiment when the animals deprived of ascorbic acid showed signs of scurvy, all the animals were fasted overnight and the next morning blood was collected by cardiac puncture without anesthesia. Total cholesterol(8), NEFA(9), phospholipid(10), lipoproteins(11) and triglycerides(12) of plasma were estimated. Triplicate unstained plasma samples were run parallel to stained plasma samples in the paper electrophoresis apparatus and the zones corresponding to the beta-lipoproteins were cut into small pieces. Cholesterol in the paper pieces was eluted with warm alcohol-acetone mixture (1:1) and the eluted samples were evaporated to dryness. The residue was used for estimation of cholesterol(8).

Results. The results are given in Table I. Total plasma cholesterol did not change appreciably in the scorbutic guinea pigs. Ascorbic acid supplementation reduced the cholesterol level below normal. Beta-lipoprotein cholesterol which was increased markedly in scurvy returned to normal on ascorbic acid supplementation but remained unaltered after insulin treatment. The altered lipoprotein pattern in scurvy became normal when the scorbutic animals recovered after the ascorbic acid treatment and did not change

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TABLE I. Difference in Fractions of Plasma Lipids of Guinea Pigs Under Different Treatments.

Condition of the animals	Total cholesterol (mg %)	Beta lipoprotein cholesterol (mg %)	Lipoproteins: Alpha (%)	Lipoproteins: Beta (%)	Phospholipid as lecithin (mg %)	Triglyceride (mg %)	NEFA (micro-equivalent per liter)
Normal	66 ± 3.39 (10)	32 ± 5.28 (8)	39.6 ± 3.50 (5)	60.4 ± 3.74 (5)	50 ± 6.40 (8)	24.80 ± 2.98 (7)	312 ± 49.80 (8)
Scorbutic	72 ± 9.67 (7)	49.60 ± 3.78 (8)	16.8 ± 3.65 (5)	83.20 ± 3.95 (5)	78 ± 11.68 (7)	11.72 ± 1.50 (8)	740 ± 58.60 (10)
Recovered from scurvy	50 ± 6.50 (7)	32.60 ± 3.57 (7)	42.50 ± 2.17 (8)	57.50 ± 1.68 (8)	75 ± 8.24 (7)	25.57 ± 4.64 (7)	350 ± 68.91 (7)
Insulin treated scorbutic	77 ± 9.07 (7)	42.70 ± 10.44 (6)	21 ± 4.52 (4)	79 ± 1.96 (4)	70 ± 5.77 (7)	24.29 ± 1.02 (5)	403 ± 55.38 (5)

Values are mean ± S.E. Figures in parentheses indicate No. of animals.

after insulin treatment. Plasma phospholipids of scorbutic animals, which increased in scurvy, did not change after ascorbic acid or insulin treatment. Plasma triglycerides diminished considerably in scurvy. The values could be brought back to normal by both insulin treatment and ascorbic acid supplementation. Plasma NEFA increased considerably in scurvy and could be reduced to normal by either insulin treatment or ascorbic acid supplementation.

Discussion. The results show considerable alteration in the different fractions of plasma lipids in scurvy. Along with the increase in plasma beta lipoproteins in the scorbutic guinea pigs there had been a simultaneous increase in beta lipoprotein-cholesterol. These changes could be turned to normal by ascorbic acid supplementation, indicating a possible role of ascorbic acid as a hypocholesteremic agent. The significant decrease in plasma cholesterol level of animals recovered from scurvy after ascorbic acid supplementation as compared to normal animals further supports the hypocholesteremic function of ascorbic acid. Since increased beta lipoprotein-cholesterol is considered to be one of the important indices to assess the degree of atheromatous lesions, it is pertinent to suggest that ascorbic acid deficiency may be associated with metabolic defects in the arteries.

The increased plasma NEFA in scurvy was not due to inanition as the normal control animals received the diet consumed by its scorbutic pair. It is probably due to the associated abnormal glucose tolerance and insulin insufficiency(5). It has been shown in this laboratory (unpublished data) that the abnormal glucose tolerance of scorbutic guinea pigs can be corrected by ascorbic acid supplementation. The decrease in plasma NEFA values in animals recovered from scurvy after ascorbic acid supplementation might be related to the corrected carbohydrate metabolism. The decrease in plasma NEFA in animals receiving insulin treatment might be due to the action of insulin on the adipose tissue secondary to the improved glucose tolerance, with the resultant promotion of esterification of fatty acids in the adipose tissue(13).

We have observed earlier that total body lipid is diminished in scorbutic guinea pigs and that insulin treatment has a beneficial effect(14). The decreased plasma triglycerides in scorbutic guinea pigs might be due to combined effects of decreased total body lipid and increased mobilization of NEFA from fat depots. Insulin and ascorbic acid corrected the diminished plasma triglycerides probably by increasing lipid synthesis(14) and by re-esterifying the fatty acids.

The role of plasma phospholipids in scurvy is rather obscure. The increased value obtained in scurvy could not be corrected either by insulin treatment or by ascorbic acid supplementation. Since lipid synthesis is decreased in scurvy, the available fatty acids are transported more as phospholipids. After insulin treatment or ascorbic acid supplementation, though there was increased fat synthesis it is possible that there was diversion of fatty acids towards phospholipid formation in a preferential way. Insulin and ascorbic acid seem to have no specific role in the level of plasma phospholipids.

Summary. The different fractions of plasma lipids were estimated in normal, scorbutic, insulin treated scorbutic and ascorbic acid supplemented scorbutic guinea pigs. Beta lipoprotein : alpha lipoprotein ratio, beta lipoprotein cholesterol, phospholipids and NEFA of plasma were increased and plasma triglycerides diminished in scorbutic animals. While insulin treatment of the scorbutic animals corrected only the plasma NEFA and triglyceride values, ascorbic acid supplementation brought back all these values to normal levels. Total plasma cholesterol did not change in scurvy. Ascorbic acid supplementation lowered the plasma cholesterol value below the value seen in normal guinea pigs, indicating hypocholesteremic effect of ascorbic acid. Neither insulin nor ascorbic acid seem to have specific roles in the metabolism of phospholipids.

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Low Values for Sebum in Eunuchs and Oöphorectomized Women.* (28050)

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Androgenic stimulation has been shown to induce acne and is believed to be the incitant of this condition in the human male(1,2). Testicular secretions seem to be the usual source of acne-inciting androgens in males, since unpublished observations showed that acne did not develop after castration in any of 91 males. Testicular secretions and androgenic treatment enhance sebaceous secre-

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