Vitamin C Effect of an Oxidized Diet[1] (35766)

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(Introduced by F. W. Dunihue)

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As investigations over many years indicate, and study on scurvy by Fullmer *et al.* support (1), the role of ascorbic acid in the body economy and in the prevention of scurvy is most likely a multiple one. Probably the most agreed upon function of ascorbic acid is its part in the hydroxylation reactions concerned with collagen formation. In this case the mechanism of action of ascorbic acid is probably through free hydroxyl radical formation (2). According to Robertson (2) and Gould (3), collagen may be formed in small amounts at a slow rate in the absence of ascorbic acid, and the possibility exists that sources other than ascorbic acid may supply the free hydroxyl radical. We recently noted that a delay in the onset of scurvy in guinea pigs resulted from the inadvertent use of a batch of outdated ascorbic acid-free test diet. Since the diet had a rancid odor, we hypothesized that dietary or tissue lipid peroxidation, through the formation of free radicals (4), might substitute, at least in part, for ascorbic acid and delay scorbutogenesis. If experimentally demonstrated, this would provide tangible support for Robertson's hypothesis (2). Observations provided in this paper do lend such support.

Materials and Methods. Male English short hair guinea pigs, weighing about 175 g, were acclimated for 5–7 days to a nonrancid pelleted ascorbic acid-free synthetic guinea pig test diet (5) supplemented with cabbage and 2 mg of orally administered ascorbic acid every day. After this period, all healthy growing animals were paired by weight into the following groups for the duration of the experiment: Group 1 continued to receive the nonrancid, ascorbic acid-free test diet. Group 2 was given the same diet but one which had become oxidatively rancid (confirmed by infrared analysis (6)); Group 3 also received the rancid, ascorbic acid-free diet plus 30 mg of alpha tocopherol acetate administered orally three times a week; Group 4, an *ad libitum* control group received the nonrancid, ascorbic acid-free diet plus 50 mg of ascorbic acid administered orally three times a week; Group 5 was a pair-fed control group, each animal receiving daily the average food intake of Group 1 plus 50 mg of ascorbic acid in the same manner as Group 4.

Animals were housed individually in separate cages and a record was kept of their weight, food consumption, and general appearance three times a week. After 16 or 17 days, the animals were tagged by a code unknown to the examiners and presented to them in a mixed random fashion for gross examination. Each animal was decapitated and its blood was collected into a heparinized tube. The animals were then autopsied. Special attention was given to hemorrhaging at the wrist and tibial joints; subcutaneous hemorrhage; bleeding into the alimentary tract, lungs, and costochondral junctions; and condition of the incisors. Beading at rib junctions was also looked for. The condition of the internal organs was noted and the adrenal glands were measured. Selected tissues were removed for histological examination. Decoding of animal groups and assembly of examination findings were done after all observations were made.

Alkaline phosphatase activity was measured in the plasma by a modification (7) of the Bessey *et al.* (8) method. Each animal

[1] Supported by U. S. Public Health Service Grant AM-10984 and Hoffmann-LaRoche Inc.

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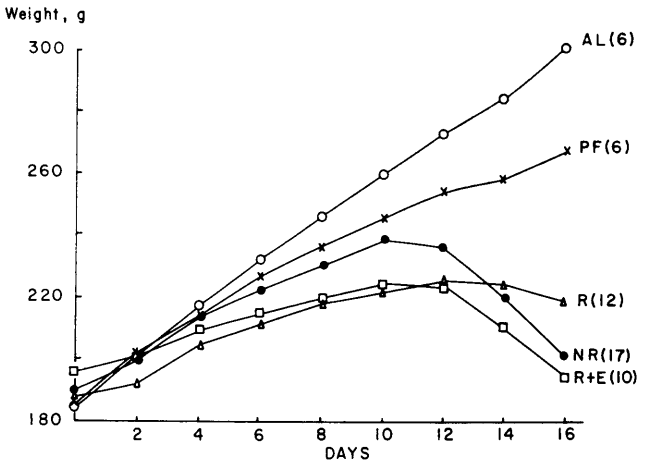

FIG. 1. Growth curves of guinea pigs in the following test and control groups: (AL) Group 4, *ad libitum* controls; (PF) Group 5, pair-fed controls; (R) Group 2, rancid, ascorbic acid-free diet; (NR) Group 1, nonrancid, ascorbic acid-free diet; (R + E) Group 3, rancid, ascorbic acid-free diet plus alpha tocopherol. The number of animals per group is given in parenthesis.

was classified independently by the authors as having no, borderline, slight, moderate, or severe scurvy with the assignment of a value of 0, 0.5, 1, 2, and 3, respectively. To arrive at the degree of scurvy for the group, the values were totaled and divided by the number of animals in the group.

Results. The results are presented in Fig. 1 and Table I. Results for animals on the experiment for 16 days were essentially the same as those for the animals in the two experiments lasting 17 days and it was considered reasonable to consolidate the results. As shown on the growth curves (Fig. 1), all three groups without ascorbic acid attained their maximum average weight at 10–12 days. The average loss of weight during the next 4 days was 32 g for the animals in Group 3 (ascorbic acid-free nonrancid diet), 26 g for those in Group 5 (ascorbic acid-free, rancid diet plus alpha tocopherol acetate) but only 9 g for the animals in Group 3 (ascorbic acid-free, rancid diet).

Two authors each independently assigned identical ratings for the degree of scurvy in 40 of 51 animals. In the remaining 11 cases, they differed by 1 category and in these instances the data were reexamined and agreement was reached on an evaluation. Average ratings for groups 1 and 3 were 2.4 (moderate to severe) and 2.0 (moderate), respectively; the difference between them was not statistically significant at the 5% level. Group 2 with an average rating of 1.0 (slight) was significantly different at the 5% level from both Groups 1 and 3. All animals receiving ascorbic acid were ascorbutic.

The adrenal glands for all three ascorbic acid-free groups were enlarged compared to *ad libitum* and pair-fed controls. Alkaline phosphatase values were lowered in all groups not receiving ascorbic acid but least so in Group 2 animals on the rancid diet.

Discussion. Animals on the rancid, ascorbic acid-free diet were clearly much less scorbutic than animals on the nonrancid, ascorbic acid-free diet as manifest by healthier appearance, delayed and decreased weight loss, more efficient utilization of food, and fewer anatomic findings at autopsy. Of the three ascorbic acid-free groups, alkaline phosphatase activity, which decreases in scurvy (9, 10), was highest in the group with the rancid, ascorbic acid-free diet. Values for individual animals approached the normal range.

On the other hand, animals on the rancid, ascorbic acid-free diet plus alpha tocopherol were as severely scorbutic as those on the nonrancid, ascorbic acid-free diet. These re-

TABLE I. Animal Data Chart and Incidence of Degrees of Scurvy.

Diet	Wt (g)			Total food consumption (g)	Units plasma alkaline phosphatase	Incidence[c] of extent of scurvy					Severity value[d]
	Initial	Final	Gain			No scurvy	Borderline	Slight	Moderate	Severe	
Test groups (no ascorbic acid)											
1. Nonrancid (17)[a]	189 ± 7[b]	196 ± 10[b]	7 ± 10[b]	174 ± 11[b]	1.55 ± 0.21[b]	0	0	2	7	8	2.4 ± 0.2[b]
2. Rancid (12)	188 ± 9	220 ± 10	32 ± 28	158 ± 12	2.30 ± 0.52	1	3	5	3	0	1.0 ± 0.2[e]
3. Rancid + α-tocopherol (10)	196 ± 8	190 ± 9	−6 ± 11	163 ± 7	1.04 ± 0.41	1	1	1	3	4	2.0 ± 0.4
Control Groups (with ascorbic acid)											
4. *Ad libitum* (6)	184 ± 18	307 ± 22	124 ± 20	230 ± 20	8.68 ± 0.88	6	0	0	0	0	0
5. Pair-fed (6)	186 ± 20	265 ± 22	79 ± 13	194 ± 13	7.35 ± 0.84	6	0	0	0	0	0

[a] Number in parentheses is the number of guinea pigs in the group.

[b] Mean ± SEM.

[c] Number of animals.

[d] To calculate severity value, the incidence for each category of extent of scurvy is multiplied by the assigned value for the category. Then the sum of these numbers for all categories is divided by the total number of animals in the group. Assigned values: no scurvy = 0; borderline = 0.5; slight = 1.0; moderate = 2.0; severe = 3.0.

[e] Different from Groups 1 and 3 (.05 level).

sults were anticipated since alpha tocopherol is a lipid antioxidant and inhibitor of free radical chain reactions (11) and thus could negate an antiscorbutic effect due to free radical formation from lipid peroxidation in the rancid diet.

The finding of antiscorbutic activity in a synthetic diet containing peroxidized lipid but no ascorbic acid coupled with the knowledge that, under certain conditions, ascorbic acid is known to favor lipid peroxidation (12–15) suggest the possibility of an ascorbic acid–lipid interrelationship in the mechanism for the antiscorbutic action of ascorbic acid. These results also suggest that substances such as Cu^+ (16), lipoic acid (17), dichlorophenolindophenol, and methylcholanthrene (1, 18), claimed to have limited antiscorbutic activity, might be reinvestigated as possible free radical formers or as pro-oxidants of lipids.

Summary. Guinea pigs on an oxidatively rancid, ascorbic acid-free test diet showed a lesser degree of scurvy when compared to overtly scorbutic animals on the nonrancid ascorbic acid-free test diet. Simultaneous administration of alpha tocopherol, an antioxidant and free radical scavenger, to guinea pigs on the rancid, ascorbic acid-free diet blocked the antiscorbutic effect. It is postulated that lipid peroxidation may act antiscorbutically by supplying the free hydroxyl radical ordinarily arising in the presence of ascorbic acid.

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Received April 1, 1971. P.S.E.B.M., 1971, Vol. 137.