Effect of Dietary Protein and Ascorbic Acid Levels on Biosynthesis of Collagen.* (26461)

L. A. BAVETTA, PAT O'DAY AND ISAAC BEKHOR
Department of Biochemistry and Nutrition, School of Dentistry, University of Southern California, Los Angeles

An important relationship has long been implicated between ascorbic acid and the metabolism of collagen and connective tissue. Hojer(1) reported that in scurvy, collagen formation is diminished and there is a deficiency of collagen in the connective tissue. Abt *et al.*(2) as well as Schauble *et al.*(3) reported that, in guinea pigs, a direct relationship exists between the amount of ascorbic acid in the diet and its concentration in the scar tissue. Gould(4) has demonstrated a direct effect of ascorbic acid on rate of biosynthesis of collagen in the guinea pig. In addition, the literature is replete with experimental studies relating the importance of Vit. C to normal wound healing. Although a need for ascorbic acid in collagen formation is quite evident, the exact mechanism is not entirely clear.

It is well known that rats synthesize ascorbic acid under normal conditions to meet their metabolic needs. However, it is possible that under conditions of stress, such as a low protein diet, synthesis of ascorbic acid or its utilization may be impaired so that rate of collagen biosynthesis is affected.

This experiment was designed to determine the rate of collagen biosynthesis in rats under the influence of different dietary levels of protein with and without extra ascorbic acid supplementation. The biosynthesis of collagen was determined utilizing the sponge technic as employed by Boucek(5). It was assayed at the end of 7, 14, and 21 days after implantation. Collagen was quantitatively estimated by measuring the hydroxyproline content after hydrolysis employing the method of Newman and Logan(6).

Methods. The diets employed contained casein at the 8% and 22% level. The basal ration had the following composition: casein† 8.0; sucrose, 70.5; cottonseed oil, 10.0; Wesson salt mix, 5.0; choline chloride, 0.2; and l-cystine, 0.3. To each kg were added the following crystalline vitamins: thiamine hydrochloride, 40 mg; riboflavin, 20 mg; pyridoxine hydrochloride, 20 mg; calcium pantothenate, 100 mg; nicotinic acid, 100 mg; ascorbic acid, 200 mg; biotin, 4 mg; folic acid, 10 mg; para-aminobenzoic acid, 400 mg; inositol, 800 mg; Vit. B_{12}, 150 μg; 2-methylnaphthoquinone, 5 mg; choline chloride, 2 g; Vit. A, 5000 U.S.P. units; Vit. D_2, 500 U.S.P. units; and alpha tocopherol acetate, 100 mg. One of the 8% casein diets and one of the 22% casein diets had 1 g of ascorbic acid added to each kilogram of diet in addition to the amount of ascorbic acid already present in the vitamin mixture employed (0.2 g ascorbic acid per kg of diet). All diets were prepared fresh every 3 days and stored in a refrigerator at 4°C.

One hundred and twenty young male rats of the Holtzman strain, weighing 90 to 110 g were used. The animals were divided into 4 groups of equal weight distribution and each group was placed on one of the above diets. The animals were housed in individual wire bottomed cages and were allowed to eat and drink *ad lib.* during the experiment. All animals were weighed twice weekly.

After one week on their respective diets the rats were implanted with the polyvinyl sponges. One sponge weighing from 100-140 mg was implanted aseptically along the dorsum of each rat. One-third of each group was sacrificed after 7, 14 and 21 days respectively after sponge implantation. The sponge tissue was removed from each animal along with samples of dorsal and ventral skin. Each animal had been shaved with electric clippers to remove hair from the skin areas where the skin samples were taken. All these samples were immediately frozen subse-

* This investigation was supported by U.S.P.H.S. grants.

† Vitamin-free Test Casein, General Biochemicals, Chagrin Falls, Ohio.

TABLE I. Influence of Excess Ascorbic Acid on Biosynthesis Sponge Collagen in the Rat.

	8% casein groups						22% casein groups					
	Wk 1		Wk 2		Wk 3		Wk 1		Wk 2		Wk 3	
	With Vit. C	Without Vit. C	With Vit. C	Without Vit. C	With Vit. C	Without Vit. C	With Vit. C	Without Vit. C	With Vit. C	Without Vit. C	With Vit. C	Without Vit. C
No. of animals	10	8	9	9	7	8	10	10	10	9	9	9
Mean sponge collagen value (mg collagen/g sponge)	37.2	24.0	66.4	61.1	99.0	103.0	66.4	45.7	112.4	85.5	142.3	136.4
Standard deviation	14.6	7.8	12.6	12.6	24	21	19.3	10.1	21.2	13.2	22.4	25.4
Standard error	4.6	2.8	4.2	4.2	9.1	7.5	6.1	3.2	6.7	4.4	7.48	8.46
Difference between Means	13.2		5.3		4.0		20.7		26.9		5.9	
SE_D	5.35		5.9		11.8		6.87		8.0		11.2	
T value	2.5		.9		.34		3.02		3.35		.53	
P "	.02		>.1 <.5		>.5		<.01 >.001		<.01 >.001		>.5	

quent to collagen analysis.

Results. The results of the biosynthesis of collagen in the sponge implants are given in Table I along with the statistical data. After one week the 8% casein group with extra Vit. C added to its diet had a mean value of 37.2 mg collagen per g implanted sponge whereas the 8% casein group without the additional Vit. C had 24.0 mg of collagen per g implanted sponge. The P value between the groups was 0.02, thus there is a significant difference between the collagen values indicating the added Vit. C stimulated collagen biosynthesis during this period. After 2 weeks the 8% casein group with added Vit. C had a mean sponge collagen content of 66.4 mg of collagen per g sponge and the 8% casein group without added Vit. C supplementation had an average content of 61.1 mg per 1 g sponge. There is probably no significant difference between the two groups at the end of the second week or at the end of 3 weeks.

One week after sponge implantation, the 22% casein group with added Vit. C supplementation had a mean sponge collagen content of 66.4 mg collagen/g sponge, while the corresponding group without added Vit. C had a value of 45.7 mg collagen/g sponge. This represents a highly significant difference between the 2 groups. After 2 weeks the Vit. C supplemented group had an average sponge collagen content of 112.4 mg collagen/g sponge whereas the unsupplemented 22% group had a mean value of 85.5 mg collagen/g sponge, a highly significant difference. There is no significant difference between the two groups at 3 weeks. Thus there is a significant increase in biosynthesis of sponge collagen mediated by Vit. C supplementation at the first week with a 8% casein diet and at both the first and second week on the 22% casein diet. The content of collagen in skin of the animals from the various experimental groups is given in Table II. No essential difference between the groups was found. No significant differences were observed in weight increment of the various groups which could be ascribed to supplementation with extra amounts of Vit. C.

TABLE II. Skin Collagen Expressed as Percent Collagen Dry Fat Free Sample.

Casein	Wk 1		Wk 2		Wk 3	
	With Vit. C	Without Vit. C	With Vit. C	Without Vit. C	With Vit. C	Without Vit. C
8%	33.5	35.0	42.0	37.3	42.4	43.2
22%	34.0	32.9	37.2	34.6	37.7	39.4

Discussion. The results of these experiments indicate a direct action of ascorbic acid on the initial biosynthesis of collagen in implanted polyvinyl sponges in the rat. This effect was much more marked under conditions of higher protein intake. It is quite probable that the mechanism for rapid collagen formation requires ascorbic acid as a component or that the vitamin may serve to expedite earlier release of some inhibitory mechanism resulting in rapid biosynthesis of collagen. The negative results obtained on skin collagen may be explained on the basis of the extremely slow turnover of body collagen as compared to such rapid synthesis as that encountered in the experimental system employed.

Summary. Our results indicate that on a diet containing 22% casein, extra amounts of dietary ascorbic acid caused a substantial increment in collagen synthesis which was significant during the first 2 weeks after sponge implantation. It was also increased by Vit. C supplementation of diets containing 8% casein but the results were less marked than with the higher protein level. The results also indicate that addition of extra amounts of Vit. C to these diets improved their biological value from the point of view of biosynthesis of collagen but not for growth.

1. Hojer, J. A., *Acta Paidiat.*, 1923-1924, v3, suppl. 3, 1.

2. Abt, F. A., Schuching, von S., Roe, H. J., *J. Nutrition*, 1960, v70, 427-437.

3. Schauble, J., Chen, R., Postlethwait, R. W., Dillon, M. L., *Surgery, Gynecology, and Obstetrics*, 1960, v110, 314-318.

4. Gould, S. B., *J. Biol. Chem.*, 1958, v232, 637.

5. Boucek, R. J., Nobel, N. L., *A.M.A. Arch. Path.*, 1955, v59, 553.

6. Neuman, R. E., Logan, M. A., *J. Biol. Chem.*, 1950, v184, 299.

Received February 15, 1961. P.S.E.B.M., 1961, v106.

Countercurrent Exchange in Vessels of Renal Medulla. (26462)

NEILS A. LASSEN* AND JAMES B. LONGLEY
(Introduced by G. L. Laqueur)
Laboratory of Pathology and Histochemistry, National Institute of Arthritis and Metabolic Diseases, Bethesda, Md.

After the proposal of the countercurrent multiplier hypothesis of renal medullary function, Wirz(1) was able to show by micropuncture experiments that the papillary blood supply was subject to renal countercurrent osmotic concentration and dilution. Berliner *et al.*(2) in restating and modifying the countercurrent hypothesis considered the medullary circulation to be simply a passive countercurrent exchange system working in parallel with an active tubular countercurrent system. Gottschalk and Mylle(3) have concurred in this view, and presented data confirming Wirz's original observations.

Where a gradient for any diffusible material exists between its 2 ends, a countercurrent exchanger acts as a barrier to the net transport of that material along its long axis. The more diffusible the material, the more effective the barrier. In a passive system concentrations of sodium, chloride, and urea, the principal elements of the papillary hy-

* Present address: Bispebjerg Hospital, Copenhagen, Denmark.