and UDPG) were not altered in amount. These compounds might be expected to be relatively slowly attacked by a phosphatase.

On the basis of these experiments, a procedure has been selected which allows intact tissues to be killed and phosphate esters extracted with a minimum of phosphatase-catalysed hydrolysis[5].

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D-Ascorbic acid and collagen synthesis

Although D-ascorbic acid has long been considered ineffective as an anti-scorbutic agent[1], BURNS *et al.*[2] reported that life and weight of guinea-pigs could be maintained and some scorbutic symptomatology prevented by giving D-ascorbic acid frequently enough to compensate for its rapid excretion. Since the animals still had hemorrhagic joints and abnormal predentin, these investigators concluded that D-ascorbic acid can replace some of the non-specific, anti-scorbutic activity of L-ascorbic acid, if its concentration in the tissues is maintained but that some activities of L-ascorbic acid are specific. The determination of whether larger or more frequent dosage of the D-isomer could prevent the characteristic and classical symptom of vitamin C deficiency, namely, an inability to form collagen during tissue repair[3,4], might help to delimit the possible mechanisms of ascorbic acid activity in connective-tissue metabolism. This paper reports the ability of D-ascorbic acid to promote collagen synthesis in the scorbutic carrageenan granuloma in guinea-pigs when administered in large doses twice daily.

The subcutaneous injection of carrageenan into guinea-pigs induces within 14 days formation of a massive granuloma. About 12% of the dry weight of the granuloma is collagen if the animals receive more than 5 mg of vitamin C daily; however, if the animals are fed a scorbutigenic diet for the 14 days, then a "scorbutic" granuloma is obtained containing about 2% collagen. Administration of L-ascorbic acid to the guinea-pigs at this time initiates rapid collagen synthesis and within 3 days the concentration in these "recovery" granulomas approaches 12% (see ref. 5). A direct proportionality between collagen and ascorbic acid concentration of granulomas has been demonstrated up to an ascorbic acid concentration of 50 μg/g (see ref. 4).

Since large amounts of D-ascorbic acid were to be used in this system, it was

TABLE I

ACTIVATION OF MYROSULPHATASE BY L-ASCORBIC ACID AND D-ASCORBIC ACID

To 5 ml of 6 mM Sinigrin in 5 μM NaCl and 0.5 mM phosphate at 30° was added activator to the appropriate concentration. After neutralization, 1 ml of enzyme solution was added. A pH of 7.0 was maintained with a pH-stat and the rate of NaOH addition measured.

Activator	*Sinigrin hydrolysed (μmoles/ml)*
None	0.17
L-Ascorbic acid (2 mM)	68.0
L-Ascorbic acid (1 μM)	0.60
D-Ascorbic acid (10 mM)	0.38

TABLE II

ASCORBIC ACID CONCENTRATION IN ADRENALS AND GRANULOMAS

Guinea-pigs were injected subcutaneously with 6 ml of 1 % carrageenan and placed on a scorbutigenic diet. Treatment was begun on the 14th day and continued until the 17th day when the animals were killed.

Treatment	*Number of animals*	*Ascorbic acid*	
		Adrenals (mg/g)	*Granuloma (μg/g)*
None	4	0.04	9
L-Ascorbic acid (100 mg/day)	6	1.56	332
D-Ascorbic acid (100 mg/12 h)	6	1.29	216

necessary to assure ourselves of the absence of racemization during synthesis. ETTLINGER *et al.*[6] have reported that the rate of hydrolysis of sinigrin catalyzed by a myrosulphatase (EC 3.1.6.5, also called glucosinolase) from mustard seed is greatly increased by L-ascorbic acid but only slightly by D-ascorbic acid. This enzyme was applied to measure L-ascorbic acid in the D-ascorbic acid used. D-Ascorbic acid synthesized according to SALOMON, BURNS AND KING[7] had m.p., 185° (uncorr.); $[\alpha]_{5890}^{24^\circ}$ −47.4°; and a purity of 95 % as determined by titration with dichlorophenolindophenol. When tested with myrosulphatase prepared according to ETTLINGER *et al.*[6], the maximum contamination with L-ascorbic acid was found to be less than 1 in 10000 (Table I).

Scorbutic granulomas were produced in male guinea-pigs weighing 300 g. Beginning on the 14th day, 6 guinea-pigs were injected intraperitoneally with 100 mg neutralized D-ascorbic acid at 12-h intervals and were killed 12 h after the sixth injection. 6 guinea-pigs were injected intraperitoneally with 100 mg L-ascorbic acid daily and were killed 24 h after the third injection. 4 guinea-pigs remained on the scorbutigenic regimen without supplement and were killed on the 17th day. The ascorbic acid concentration of the adrenals and granulomas was determined by a slight modification of the method of ROE[8]. Collagen was extracted from the granulomas with hot 5 % trichloroacetic acid[9] and hydroxyproline determined on the hydrolysed collagen using a modification of the method of NEUMAN AND LOGAN[10].

Although the concentration of D-ascorbic acid 12 h after injection is lower than that of L-ascorbic acid 24 h after injection of the same amount (Table II), enough ascorbic acid remained in the granuloma to permit maximal collagen synthesis. As

TABLE III

COLLAGEN CONCENTRATION IN GRANULOMAS AFTER TREATMENT WITH L-ASCORBIC ACID AND D-ASCORBIC ACID

Same conditions as Table II.

Treatment	*Number of animals*	*Collagen hydroxyproline (mg/g)*
None	4	0.39 (0.26–0.65)
L-Ascorbic acid (100 mg/day)	6	2.42 (1.91–3.17)
D-Ascorbic acid (100 mg/12 h)	6	2.45 (1.97–3.15)

may be seen in Table III, the scorbutic granuloma with no treatment has its usual low concentration of collagen hydroxyproline but in those animals receiving either D-ascorbic acid or L-ascorbic acid the concentration is typical of "recovery" granulomas and equally high.

This demonstration that D-ascorbic acid repairs the fundamental defect of scurvy, impaired collagen synthesis, and the previously reported ability to maintain growth and life[2] suggest that this isomer can actually replace the specific anti-scorbutic activities of L-ascorbic acid. BURNS *et al.*[2] had come to more limited conclusions concerning the activity of D-ascorbic acid because the relatively small dosage used, 6 mg D-ascorbic acid twice daily, still permitted the appearance of some scorbutic lesions.

Probably many analogs of ascorbic acid, in addition to those already demonstrated, might function as L-ascorbic acid if adequate tissue concentrations could be maintained. This relative lack of specificity, especially stereospecificity, for ascorbic acid in mammalian metabolism makes it unlikely that this vitamin functions as a coenzyme. The non-specificity is compatible with the suggestion that ascorbic acid activity is based on its ability to generate monodehydroascorbyl and hydroxyl radicals[4].

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