

SC 2215

Changes in deoxyribonucleic acid, collagen and ascorbic acid during the development of carrageenan granuloma

The action of ascorbic acid on the biosynthesis of collagen has been assumed by ROBERTSON¹ to occur at a number of sites. Its participation in hydroxylation has been sufficiently substantiated (for a review see ref. 1). Moreover, it has been shown that in artificially enhanced fibrillogenesis there occurs an accumulation of ascorbic acid in the granulation tissue²⁻⁴; according to WOESSNER AND BOUCEK⁵ this accumulation is connected with the cell content whereas other authors find accumulation of ascorbic acid only in the later stages^{6,7}.

In view of the conflicting reports regarding connections of ascorbic acid with the individual components of connective tissue, this problem has now been studied in the course of development of carrageenan granuloma, the latter serving as a model of enhanced fibroplasia.

Carrageenan granuloma was induced in the subcutis of guinea-pigs by the procedure of ROBERTSON AND SCHWARTZ⁸. From tissue that had been isolated after various days of development of the granuloma ascorbic acid⁹ and deoxyribonucleic acid¹⁰ were extracted and after acid hydrolysis the amount of total hydroxyproline¹¹ estimated. Ascorbic acid was also determined in serum⁹.

The results of this work are presented in two ways: Fig. 1 shows changes in the substances investigated in absolute amounts in the whole granulation tissue of one guinea-pig. This way of stating results permits an estimate to be made of the rate of formation or degradation of the substances mentioned. From Fig. 1 it is evident that until the 5th day the changes in ascorbic acid and collagen proceed in a similar manner although a parallel in behaviour of ascorbic acid and DNA in this period should not be overlooked.

From the 5th to 7th day a striking increase of the amount of collagen and DNA occurs while the amount of ascorbic acid changes very slightly during this period.

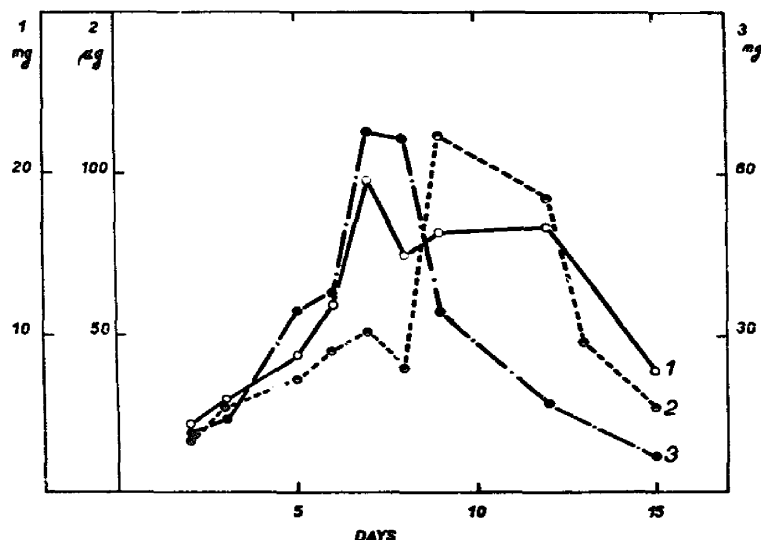


Fig. 1. Changes in the amount of ascorbic acid (O---O), DNA (●---●) and collagen (O—O) in the whole granuloma tissue in one animal. Each point represents the average value of several parallel determinations executed in two independent samples of granuloma tissue.

Only from the 8th to 9th day a pronounced increase in concentration of ascorbic acid takes place at a time when the amount of DNA decreases very markedly and the amount of total collagen proteins remains practically unchanged. At the time of the degradation of collagen between the 12th and 15th day a parallel decrease in ascorbic acid occurs.

The connection between changes in ascorbic acid and collagen are still more striking upon consideration of concentration changes (Fig. 2).

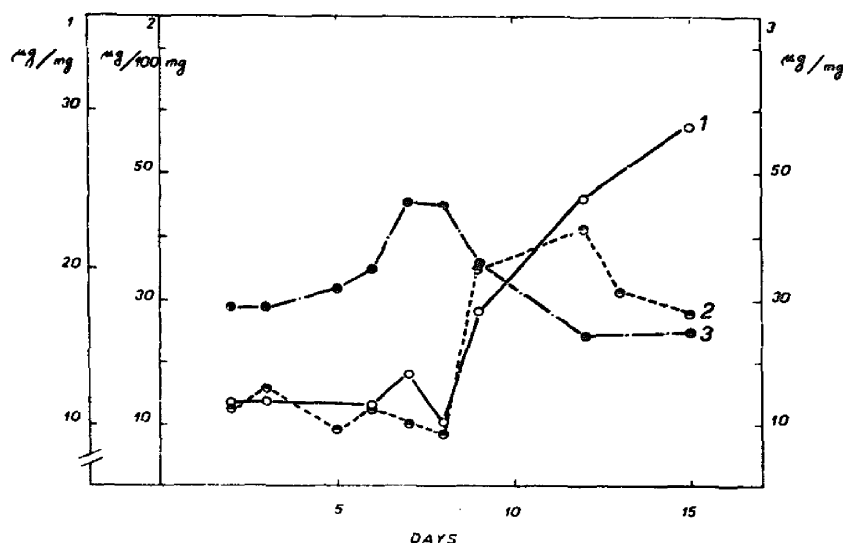


Fig. 2. Changes of ascorbic acid (●---●), DNA (●---●) and collagen (○---○) concentration in granuloma.

An approximate calculation of the change of the ascorbic acid level in the extracellular fluid of the granulation tissue suffices to exclude the possibility of ascorbic acid accumulation being due to the possible change of the extracellular fluid content of the granuloma.

The mutual relation between the individual factors during the whole development of granuloma was assessed statistically by calculating correlation coefficients. Into this statistical computation (Table I) were included the experimental data of WOESSNER AND BOUCEK⁵ and our previous results dealing with similar problems¹².

TABLE I

CORRELATION COEFFICIENTS (r) OF INTERRELATION BETWEEN ASCORBIC ACID, DNA AND COLLAGEN

	Ascorbic acid collagen	Significance P	Ascorbic acid DNA	Significance P
WOESSNER AND BOUCEK ⁵	+0.80	(< 0.01)	+0.82	(< 0.01)
ČMUCHALOVÁ AND CHVAPIL ¹²	+0.68	(< 0.05)	-0.12	(insignificant)
Present data (μg/unit dry wt.)	+0.81	(< 0.01)	-0.67	(insignificant)
Present data (μg/granuloma of one animal)	+0.65	(< 0.01)	+0.04	(insignificant)

The results of statistical treatment show that regardless of the manner of expressing results ascorbic acid changes during development of carrageenan granuloma correlate significantly with collagen only. Only in the experiments of WOESSNER AND BOUCEK with an implanted polyvinylchloride sponge does ascorbic acid correlate equally significantly with cells as well as with collagen.

The established connection between changes of ascorbic acid and collagen supports the findings of SINGH *et al.*⁶ reporting an accumulation of ascorbic acid in the callus of a healing bone fracture only after a sufficient accumulation of collagen in form of the osteid. Also POSTLETHWAIT *et al.*⁷ find a pronounced elevation of ascorbic acid in the course of healing of a cut tendon or muscle at a relatively late phase of the healing, *i.e.* between the 21st and 42nd day, when it can be assumed that the cellular reaction had already acquiesced.

A further finding follows from Fig. 1. Accumulation of collagenous fibrotic tissue takes place at a time when, theoretically, a maturing of collagenous structures may be expected. Ascorbic acid would then participate also in the control of the structural stability of the collagenous fibers.

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