Short Communication

THE REACTION OF SEMICARBAZIDE WITH COLLAGEN

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When experimental lathyrism is induced in rats by administering certain aminonitriles or hydrazine derivatives two major effects observed are increased fragility and increased extractability of collagen in skin and other connective tissues (Levene and Gross, 1959). Abundant collagen is synthesised in the skin of lathyritic rats but it does not mature in the same way as in normal animals, as judged by comparing the sub-unit composition of neutral-salt-soluble and acid-soluble collagens from normal and lathyritic animals (Martin, Gross, Piez and Lewis, 1961; Martin and and Goldhaber, 1963; Martin, Piez and Lewis, 1963). Moreover, although collagen fibrils can be reconstituted, *in vitro*, from solutions of neutral-salt-soluble collagen of lathyritic rats, they are less stable than those formed in a similar way from the neutral-salt-soluble collagen of normal rats (Gross, 1963). It has been suggested that lathyritic agents act by altering the aggregation stage of collagen fibrogenesis (Smiley, Yeager and Ziff, 1962; Gerber, Gerber and Altman, 1962; Witschafter and Bentley, 1962), possibly by inhibition of cross-linking reactions (Martin and others, 1961, 1963). Evidence has been put forward for the direct action of lathyritic agents on collagen during maturation (Levene, 1962; Martin and Goldhaber, 1963). This paper reports preliminary observations on the effect of treating collagen *in vitro* with one lathyritic agent, semicarbazide (Levene, 1961). Its effect on fibril forming properties and sub-unit composition has been measured.

Neutral-salt-soluble collagen was extracted from the skins of 4–6 week old rats with 1·0M sodium chloride and purified (Wood, 1962). Solutions (0·1 per cent) of this material in 0·1M sodium chloride adjusted to pH 4·1 with 0·005M acetate buffer (Wood and Keech, 1960) were treated for known times at 35° with an equal volume of 0·15M phosphate buffer at pH 7·0 and 1/5 volume of 0·5M semicarbazide hydrochloride adjusted to pH 7·0 with sodium hydroxide. In control mixtures 0·5M sodium chloride was substituted for the semicarbazide solution. The reaction mixtures were then dialysed exhaustively against 3 per cent acetic acid and finally against 0·1M sodium chloride adjusted to pH 4·1 as above.

Fibril formation. The kinetics of fibril formation at 35°, and pH 7·0, in treated and control solutions were followed turbidimetrically (Wood and Keech, 1960). The results, of which those shown in Fig. 1 are typical, showed that treatment with the lathyritic agent retarded fibril formation. The effect was most marked on the lag period or nucleation phase (Wood, 1960). When the precipitates were cooled to 0–4° a fraction redissolved (Gross, 1958; Fessler, 1960) but the rate at which this occurred was approximately the same in treated and untreated solutions.

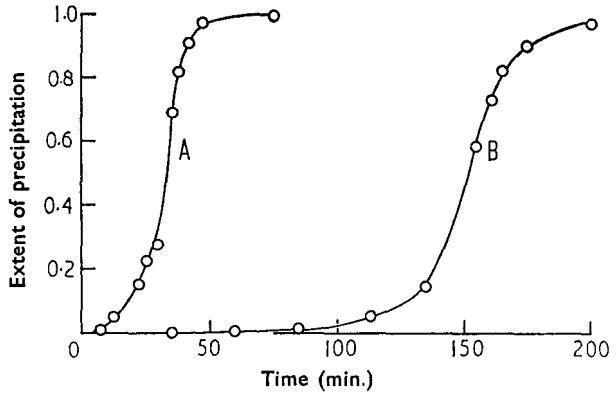

FIG. 1. Rate of precipitation at pH 7.1, 35°, I 0.23, of fibrils in solutions (0.1 per cent) of (A) neutral-salt-soluble collagen and (B) neutral-salt-soluble collagen treated with semicarbazide for 3 hr.

Sub-unit composition. Fibril formation was allowed to occur as above in solutions of treated and untreated collagen at 35° and pH 7·1 and the precipitates "aged" at 35° for three weeks (using toluene as preservative). The collagens were isolated, dissolved in 3 per cent acetic acid and dialysed against 0·15M acetate buffer, pH 4·8. The solutions (approximately 0·25 per cent) were denatured by heating to 45–50° and examined in the ultra-centrifuge at 40° (Wood, 1962). A sample of untreated neutral-salt-soluble collagen which had not been "aged" at 35° was also examined and showed the two major components (α and β) described by others (Orekhovich and Shpikiter, 1955) in relative proportions (Table I) close to those observed earlier (Wood, 1962). Both semicarbazide-treated and untreated samples which had been "aged" also showed these components but contained in addition appreciable quantities of a third, faster moving, component. The apparent relative proportions of these components (Table I) show two interesting features; (i) as suggested by earlier work (Wood, 1962) the relative proportions of the components change on ageing, the concentration of α falling, the concentrations of β and the third component rising; (ii) a similar shift is observed in "aged" semicarbazide-treated collagen but the magnitude of the effect appears to be slightly less than in the untreated sample.

TABLE I

Material	Composition (g./100 g.)		
	α	β	Third component
Neutral-salt-soluble collagen (NSS) ..	78	22	trace
"Aged" NSS	59	25·5	15·5
"Aged," semicarbazide-treated, NSS ..	63	28	9

Discussion. The results show that direct action of semicarbazide on neutral-salt-soluble collagen *in vitro* resulted in changes in the collagen somewhat similar to those observed in lathyrism. The treated collagen

formed fibrils less readily than untreated collagen although the fibrils formed were not markedly less stable than with untreated collagen. The change of sub-unit composition observing on "ageing" neutral-salt-soluble collagen may be relevant to the similar change which occurs in maturation of collagen *in vivo* and which is believed to be due to cross-linking of the polypeptide chains of the protein. In lathyrism the collagen synthesised is not as highly cross-linked as in normal animals. The present data suggest that the cross-linking, which seems to occur during "ageing," does not proceed as readily in semicarbazide-treated collagen as in untreated collagen.

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The paper was presented by THE AUTHOR.