the decolorisation is denaturation due to the breaking of hydrogen bonds or a direct action of H^+ on the binding of copper to protein is now under investigation. At present, the mode of copper binding in caeruloplasmin is unknown though both imidazole[6] and carboxyl groups[6,7] have been suggested as possibilities. To study this problem it is useful to have various means of modification of the molecule in the region of copper attachment. The reversible acid decolorisation may be of some value in this respect.

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An intermolecular defect of collagen in experimental lathyrism

The experimental disease, osteolathyrism, induced in a variety of animals by agents such as β-aminopropionitrile is characterized by mesenchymal deformities[1], loss of tensile strength, and dramatic increases in collagen solubility[2]. It has been proposed that the large pool of extractable collagen is derived from old insoluble fibrils transformed to an extractable state[2]. A contrary view holds that newly synthesized molecules are prevented from polymerizing to fibrils[3]. While the molecular dimensions, conformation and fibril-forming ability of lathyritic collagen seems not to be grossly altered[2] there is evidence for a failure of intramolecular cross-linking characteristic of the maturation process[4,5].

This preliminary report documents the observation that while extracted lathyritic collagen readily forms striated fibrils *in vitro* on warming to body temperature these fibrils fail to become insoluble with increasing time of incubation in the manner characteristic of normal extracted collagen[6].

Guinea-pigs ranging in size from 250–1500 g were injected daily into the peritoneal cavity with 0.5–1 mg of β-aminopropionitrile fumarate per g of body weight. The dosage was adjusted daily so as to induce a weight loss of approx. 1–10 g/day for 14 or more days. Young actively growing guinea-pigs were used for control material. The cleaned skins were extracted with cold 0.45 M NaCl. The extracted

collagen was isolated and purified by precipitation with an equal volume of 5 M NaCl, followed by solution of the precipitate in cold phosphate buffer (pH 7.6, I 0.4). After centrifugation to remove the small insoluble residue the supernatant solutions were dialyzed against 0.02 M disodium phosphate in the cold overnight. The fibrous precipitate was then dissolved in 0.1 M acetic acid and dialyzed extensively in the cold against the same medium prior to lyophilization. The lyophilized collagen was redissolved in phosphate to make a final concentration of approx. 0.1 %, then dialyzed against 0.4 M NaCl in the cold and centrifuged at high speed to remove any small amount of undissolved protein.

Thermal precipitation by warming 2 ml of the extracts, in triplicate, to 37° in Klett tubes for varying periods of time was monitored by measuring the opacity change in a Klett colorimeter at 530 mμ (ref. 6). All samples, both control and lathyritic, were incubated simultaneously for 4, 24, 48, 96 and 168 h, after which samples were rapidly cooled to 5° and measurements of opacity continued. Results shown in Fig. 1 and Table I were obtained with purified collagen from separate skin extracts of three lathyritic and three normal animals. The normal collagen, as previously demonstrated[6], became increasingly insoluble with time of incubation at 37°. On the other hand the lathyritic collagen dissolved almost completely in the cold even after 7 days of incubation. The same behavior was exhibited by the collagen of crude skin extracts prior to purification.

Purified normal and lathyritic collagen were compared in terms of total amino acid composition, carbohydrate content, denaturation temperature in both the dissolved and reconstituted state, optical-rotatory dispersion at 304–589 mμ, intrinsic viscosity and flow-birefringence measurements. No significant differences between normal and lathyritic collagens were found in any of these properties. Measurements of denaturation temperature at neutral pH in phosphate buffer and in the acid range

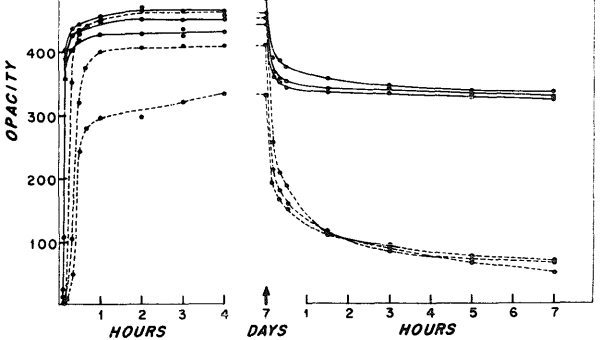

Fig. 1. Thermal gelation of NaCl purified normal (———) and lathyritic (– – – –) collagen (7 days at 37°).

in acetate buffer by both optical rotation and viscosity failed to reveal significant differences between the two. Similarly shrinkage-temperature measurements on reconstituted collagen gels at neutral pH and also on normal and lathyritic chick-embryo tendons also failed to show significant differences.

TABLE I

REVERSIBILITY OF HEAT GELATION OF COLLAGEN ON COOLING TO 3° AFTER VARIOUS PERIODS OF INCUBATION AT 37°

Time of incubation at 37° (h)	Redissolved on cooling for 7 h (%)	
	Normal	Lathyritic
4	100	100
	100	100
	100	100
24	87	100
	80	100
	78	100
48	56	100
	55	100
	54	100
96	42	97
	39	94
	33	91
168	32	89
	29	82
	26	80

It is suggested that lathyritic as well as normal collagen is essentially all in the fibrillar form in connective tissues, but that there is an increased fraction of the fibrils in lathyritic tissues which may be dissolved in cold neutral or acid buffers. In addition to a loss of intramolecular cross linking, previously formed intermolecular bonds have been disrupted, or alternatively, formation of such bonds has been blocked. The mechanism might involve a subtle alteration in collagen structure or interference by another firmly bound substance.

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