Stability of Rat Skin Collagen during Recovery from Under-Nutrition

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The growth of young rats was arrested for 6 weeks from 48 h after receiving an injection of L-[5-³H]proline. The ³H in the hydroxyproline of the newly synthesized skin collagen remained steady during under-nutrition and did not decrease during the subsequent recovery period. It was concluded that in this animal model the renewed growth did not induce degradation of the pre-existing collagen fibres.

It is now well established that the turnover of collagen in most tissues of the adult animal is extremely slow, although during growth and development of the young animal active collagen degradation can be demonstrated. This catabolic process appears to be an important controlling factor in the remodelling of the connective tissues, particularly during embryogenesis (Woessner, 1968). Studies of collagen metabolism in the embryonic-chick skin have revealed that during the period of most rapid collagen synthesis there is also an enhanced rate of collagen degradation (Woessner, 1970; Kawamoto & Nagai, 1976).

The effect of catch-up growth in young rats after a period of under-nutrition has now been examined for a number of morphological and biochemical parameters (Dickerson *et al.*, 1972; Dickerson & McNulty, 1975; McNulty & Williams, 1977). This animal model offers an opportunity to investigate the stability and integrity of the connective-tissue components when these undergo a sudden burst of renewed growth. In skin the collagen fibres are randomly orientated mainly in two dimensions and this network of fibres must expand at an even rate during growth. Therefore, using this experimental model, I selected the skin as a potentially suitable and convenient tissue for the study of possible developmental influences on collagen metabolism.

Experimental

Weanling male Sprague-Dawley rats (Carworth CFE strain) were placed on the standard pelleted diet until their weight reached 120 g (age approx. 33 days). A protein-deficient diet containing 4.5% (w/w) casein (Palmer *et al.*, 1973) was then given for 6 weeks, after which the animals were returned to the standard feed. Food and water were given *ad libitum* at all times. The animals were weighed twice weekly, and at the beginning and end of the period on the low-protein

diet and immediately before being killed by ether poisoning. Each animal was given a single intraperitoneal injection of 100 μ Ci of L-[5-³H]proline (The Radiochemical Centre, Amersham, Bucks., U.K.) in 0.5 ml of phosphate-buffered saline (Holborow & Johnson, 1967) 48 h before receiving the low-protein diet.

Groups of five or six animals were killed at selected times, and all fur was carefully removed with an electric clipper. The head, tail and feet were detached and discarded and then the whole of the remaining skin was removed. Any adhering subcutaneous fat was carefully scraped away with a blunt scalpel. Each skin was weighed and then finely minced. Replicate samples (about 1 g) were accurately weighed from each skin after thorough mixing, and hydrolysed in 50 ml of 5.7 M-HCl under reflux for 18 h. Hydroxyproline was determined by an automated method (Grant, 1964). A portion (20 ml) of each hydrolysate was evaporated to dryness on a rotary evaporator and dissolved in 5 ml of 0.1 M-HCl. Hydroxyproline was separated from proline by a modification of the chromatographic method of Moore & Stein (1954). A 2 ml sample of concentrated hydrolysate was applied to a column (1.0 cm $\times$ 18 cm) of Zeocarb 225 equilibrated with 0.067 M-sodium citrate buffer, pH 3.25, containing 8% (v/v) propan-1-ol, and the column was developed at 55°C with the same buffer. The concentration of hydroxyproline in the peak tube was accurately determined, and the specific radioactivity calculated by counting 1 ml of this eluate in 10 ml of Kennedy's (1969) scintillation fluid in a Packard liquid-scintillation counter. The total skin hydroxyproline and the total radioactivity in this hydroxyproline were calculated for each animal.

For the determination of the free proline pool, skin samples were extracted as described by Mailman & Dresden (1976), and proline was determined colorimetrically (Messer, 1961). The ³H in the proline pool was then determined on a 1 ml sample in 10 ml of Kennedy's (1969) scintillation fluid.

Results

The weight curve for rats fed on a low-protein diet for 6 weeks is shown in Fig. 1, together with the weight curve for unrestricted growth. On feeding the diet there was an initial weight loss of up to 20 g, but much of this loss was recovered by the end of the diet period. When the rats were re-fed on the pellets, growth recommenced immediately and the weight gains were very close to those by the unrestricted control group. An initial weight loss was also reported by Dawson & Milne (1976).

Fig. 1. Growth curves for rats

The test group of animals (●) was fed on a low-protein diet from the age of 33 days to 75 days and then re-fed on standard pellets. The control group (○) received the standard pelleted diet throughout. Each point represents the mean $\pm$ s.d. of 8–12 animals. The period on the low-protein diet is indicated by the horizontal bar.

Rats injected with $100\mu\text{Ci}$ of $[^3\text{H}]$ proline 48 h before being fed on the low-protein diet incorporated this into the hydroxyproline of the newly synthesized collagen. Table 1 records the data for body weight, skin weight and hydroxyproline content for the experimental groups of animals. During the period on the low-protein diet, the wet weight of the skin fell, but the collagen content, as shown by the hydroxyproline values (14%, w/w, of collagen), did not change. The ^3H incorporated initially into the total skin increased slightly between the second and last

Fig. 2. Radioactivity in skin hydroxyproline and free proline during arrested growth and subsequent recovery. Rats were injected intraperitoneally with $100\mu\text{Ci}$ of $\text{L-}[^3\text{H}]$ proline, and the total radioactivity in each rat skin hydroxyproline fraction was calculated after various time periods (●). The mean $\pm$ s.d. is shown for each plot for the same animals as in Table 1. The ^3H radioactivity in the proline pool is also given. Values were averaged from pairs of animals at each point on the graph and are given as c.p.m./g of skin ($\triangle$) and also c.p.m./whole skin ($\blacktriangle$). The period on the low-protein diet is indicated by the horizontal bar.

Table 1. Effect of arrested growth on the hydroxyproline content of rat skin

Animals were reared to a body weight of 120 g (age 33 days); they were then given a low-protein diet for 42 days. This period of under-nutrition was followed by re-feeding the standard pellets. Each value represents the mean $\pm$ s.d. for the respective group of animals.

Feeding regimen	Time on diet or pellet re-feeding	Number of animals	Body wt. (g)	Skin wet wt. (g)	Skin hydroxyproline (mg)	Specific radioactivity of ^3H in skin (c.p.m./mg of hydroxyproline)
Low-protein diet (arrested growth)	2	5	110 ± 5	13.8 ± 1.0	166 ± 15	3510 ± 210
	42	6	101 ± 12	5.8 ± 0.8	134 ± 21	5130 ± 1180
Pellets re-fed after	3	6	146 ± 7	10.5 ± 0.8	166 ± 8	4230 ± 260
42 days on the	7	6	164 ± 12	12.9 ± 2.0	168 ± 14	3790 ± 370
low-protein diet	14	5	229 ± 13	27.5 ± 2.1	317 ± 22	1920 ± 200
(recovery)	43	5	321 ± 13	32.2 ± 1.9	691 ± 37	880 ± 120

day of the diet (Fig. 2). When the animals were re-fed on pellets there was a large increase (1.8 times) in the skin wet weight during the first 3 days, with a smaller increase (1.2 times) in the collagen content. Six weeks after the recommencement of pellet feeding, the animals were approaching maximum body weight and there was an accumulated 5–6-fold increase in both skin wet weight and in collagen hydroxyproline content. The specific radioactivity of the skin hydroxyproline fell appreciably during the period of renewed growth, but the total ^3H radioactivity in the whole skin showed no statistically significant change during this period.

The loss of ^3H from the proline pool of rat skin is also shown in Fig. 2. The radioactivity was negligible after 28 days on the low-protein diet and therefore unlikely to influence the measurable ^3H in the skin collagen during renewed growth.

Discussion

It is now generally accepted that there exists an active turnover of the collagenous structures during growth and physiological remodelling, and a substantial part of this catabolic activity is believed to involve the dissolution of the pre-existing collagen fibres (Mailman & Dresden, 1976). Ohuchi & Tsurufuji (1970) reported that, in mice given a single intraperitoneal injection of [^3H]proline, radioactivity accumulated in the collagen hydroxyproline for 6 weeks and then showed a steady decline to about 20% of the maximum value for the whole skin. Their data supported the concept of a steady turnover of skin collagen during growth, declining at maturity. Klein & Chandrarajan (1977) have shown that the total ^3H in the hydroxyproline fraction of skin of rats labelled *in utero* is decreased by nearly 50% between the second and sixth week of life.

In studying rats with an interrupted growth pattern, it is possible to deplete the proline pool of label and then to examine the fate of the labelled fibrillar collagen during renewed and rapid growth. In the experiments reported here, however, there is no evidence to indicate the existence of a specific growth-induced catabolism of skin collagen during the period of recovery from under-nutrition. This situation could arise if the collagen fibres have become especially stable during this period of interrupted growth by the formation of an increased number of stabilized intermolecular cross-links (Bailey *et al.*, 1974). In consequence of this, the fibres would become more resistant to the enzymic mechanisms for collagenolysis (Etherington, 1977).

Since there was an overall expansion in skin area, these individual pre-existing fibres must have moved apart relative to each other and must have become diluted out by newly synthesized collagen. This could be laid down as new fibres or added circumferentially to these older fibres.

The stability of skin collagen in this animal model requires further examination to establish whether it is a consequence of the restricted growth. Other tissues in which the arrangement of collagen fibres is more rigidly controlled than in skin also require investigation to ascertain if these exhibit any growth-induced collagen catabolism.

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