

## Collagen Proteins in Rats on Long-Term Tyrosine Diet

Ochronotic arthropathy results from in vivo tanning of collagen fibres by oxidative polymers of homogentisic acid (MILCH<sup>1</sup>). BLIVAIS, ROSENBERG and KUTUZOV<sup>2</sup> produced experimental ochronotic arthropathy by feeding rats with 5–8% tyrosine in the diet for a period of 18–24 months. The present investigation was undertaken to gain some information about collagen proteins in tyrosine-fed rats.

**Methods.** 24 male Wistar rats with an initial weight of 30 g were divided into 2 groups. One group served as control and received standard Larsen diet (containing ground wheat, lucerne, dried milk, casein, cod-liver oil, butter, calcium carbonate and salt) and water ad libitum. The second group was fed with Larsen diet containing 5% of l-tyrosine. Chromatographically it could be shown that the tyrosine-fed rats had alkaptonuria and excreted large amounts of homogentisic acid in the urine.

After 12 months the animals were sacrificed and the following investigations were performed: (1) Routine histological examination. (2) Estimation of homogentisic acid oxidase in liver<sup>3</sup>. (3) Determination of the amount of collagen in skin and tail tendon soluble in cold 0.14M sodium chloride and 0.5M acetic acid<sup>4</sup>. (4) Acid-soluble collagen was isolated from pooled skins of control and tyrosine-treated groups by the methods of WOOD<sup>5</sup> and JACKSON<sup>6</sup>; (a) in this collagen solution the reconstitution of fibrils was followed by the turbidimetric method according to WOOD<sup>5</sup> (in all experiments a phosphate buffer pH 7.2, 1.025 was used, temperature for reconstitution was 30°C); (b) the same collagen solution was precipitated by warming to 37°C for varying periods of time and the turbidimetric registration was performed. After 24 h the samples were rapidly cooled to 5°C and the opacity was again measured<sup>7</sup>.

**Results.** After feeding the tyrosine-containing diet for 12 months no gross histological changes could be found. Histochemically we proved ochronotic pigment to be present in epithelial cells of renal tubuli as well as in sternal cartilage, where also focal degenerative changes could be observed. Homogentisic acid oxidase in liver of tyrosine-fed animals decreased about 50% in comparison with control animals. We could not produce extensive ochronosis, but these findings together showed that tyrosine-fed rats were continually 'overflowed' with homogentisic acid. The amount of neutral salt and acid-soluble

collagen in skin and tail tendon was not changed in the tyrosine-treated group.

The reconstitution of fibrils from acid-soluble collagen extracted from the skins of tyrosine-treated animals showed an acceleration of fibril formation (Figure 1). The first phase of turbidimetric curve (so-called lag phase<sup>8</sup> – when the smallest aggregates of collagen molecules are formed) is shortened. The reconstituted fibrils are more rapidly and easily formed from the collagen solution isolated from tyrosine-treated rats.

Results shown in Figure 2 were obtained from experiments of gel formation at 37°C for 24 h and following dissolution after rapid cooling to 5°C. The gel formed from collagen of tyrosine-treated rats dissolved to a lesser extent than the gel from normal control collagen.

These results suggest an increased formation of cross-linkages in collagen of tyrosine-treated rats which can be interpreted in terms of 'biological tanning' by polymerized homogentisic acid. This 'biological tanning' may be the first step towards more extensive degenerative changes in connective tissue.

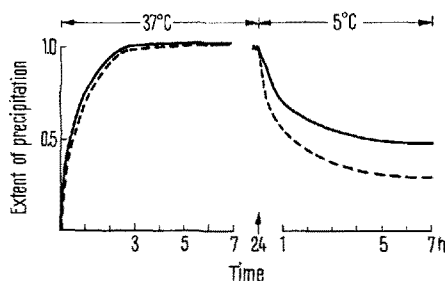


Fig. 2. Dissolution of heat formed gel from collagen on cooling to 5°C. Initial concentration of collagen 0.03%, pH 7.2. ----- = Turbidimetric curve of collagen from control; — = tyrosine treated rats. The curves represent the average of 3 measurements in each group.

**Zusammenfassung.** Bei Ratten wurde nach 12monatlicher Diät mit 5% l-Tyrosin eine Alkaptonurie hervorgerufen. Die histologische Prüfung ergab eine Übersättigung mit der Homogentisinsäure, eine verringerte Aktivität der Oxidase der Homogentisinsäure in der Leber und deren massive Ausscheidung im Harn.

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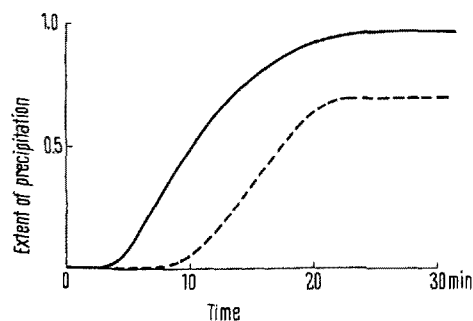


Fig. 1. Reconstitution of fibrils from collagen solution. Initial concentration of collagen solution 0.02%, pH 7.2, temperature 30°C. ----- = Turbidimetric curve of collagen from control; — = tyrosine treated rats. The curves represent the average of 3 measurements in each group.

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