Changes in Proline and Hydroxyproline in Collagens during the Development of Rats

In 1950 NEUMAN and LOGAN[1] proved that the amount of hydroxyproline in collagen proteins isolated from different mammals, as well as from avian tissues, is the same and reaches 13.4 ± 0.24 weight%. Their similar amino acid composition was further established with the aid of detailed amino acid analysis of different collagens soluble in acid, neutral or alkali medium and insoluble collagen. The ratio of proline to hydroxyproline varies between 1.5 and 1.1[2–6]. The majority of the analyses mentioned were done on collagens isolated from adult animals. Nevertheless, BOUCEK et al.[7] did not show changes in hydroxyproline concentration in collagen isolated from subcutis of 1–70 years old men, 100 days after the implantation of polyvinyl sponge. MITOMA et al.[8] found that gelatine prepared according to LOWRY et al.[9] from chick embryos or from carrageenan granuloma from subcutis of guinea pigs, contains proline and hydroxyproline in concentrations typical for commercial gelatine.

In 1956 CHVAPIL[10,11] found that, in collagen extracted by repeated extraction of lung tissue homogenate by diluted alkali[1], the amount of hydroxyproline significantly increased during the ageing of rats: collagen from 2–18 days old rats amounted approximately to 6% of hydroxyproline whereas in collagen from adult rats about 13–14% of hydroxyproline was found.

Finally, according to HALL and REED[12], the amount of hydroxyproline in collagen from skin of people from 7 to 89 years is relatively stabile with a rather decreasing trend in the course of ageing.

In this communication, we summarise our experimental proofs that in collagens prepared by two different chemical procedures, as well as in non-modified, native collagen from rat tail tendon, the amount of hydroxyproline increases especially in the early postnatal period; the content of proline does not change.

We isolated collagen proteins from lungs of six age groups of rats: 4, 9, 14, 30, 40, 60 and 240 days old:

1. by the method of LOWRY, GILLIGAN and KATERSKY[9]. Using dilute alkali extraction (0.1 N-NaOH) so that all alkali non-collagen proteins are removed and the scleroproteins (insoluble collagen and elastin) remain.

2. By the extraction of lung homogenate with boiling 0.3 *M* trichloracetic acid, according to FITCH, HARKNESS, and HARKNESS[13], we obtain all tissue collagens. In hydrolysed samples of collagen (6 N-HCl, 140°C, 3 h), we determined hydroxyproline[14], proline[15], and nitrogen[16]. In samples of scleroproteins we determined the content of elastin using the original procedure of LOWRY et al.[9].

The results of proline and hydroxyproline determination expressed in weight% of collagen or in % of protein nitrogen (Tab. I) show that the concentration of proline in insoluble collagen remains the same during ageing of rats, whereas the concentration of hydroxyproline gradually increases. For instance insoluble collagen from 4 days old rats amounts to one third of hydroxyproline content of collagen from adult animals. The ratio proline-hydroxyproline is gradually decreasing from 3:1 to magnitudes presented in literature for mature collagens (1.1–1.6). That means that during 30 days of postnatal period in rats, the collagens mature into a structure identical with collagens of adult animals as far as the content of imino acids is concerned.

There is no doubt that extraction of collagen by dilute alkali attacks its structure. GROSS[17] proved that the crystalline ultra-structure, typical for collagen, is replaced by amorphous patterns. Further, it is well known that the

Tab. I. Changes in proline and hydroxyproline content in collagen from lungs during the ageing of rats. Collagen was isolated by the method of LOWRY et al.[9]

Age of rats (in days)	Amino acid g/100 g of collagen		Proline/ Hydroxy-proline	Amino acid g/100 g N		Proline/ Hydroxy-proline
	Proline	Hydr-oxy-proline		Proline	Hydr-oxy-proline	
4	13.5	4.6	2.9	11.4	3.8	3.0
9	14.2	6.7	2.1	9.8	4.3	2.3
14	14.2	6.7	2.1	11.6	4.9	2.3
30	13.5	8.7	1.5	10.9	7.5	1.4
60	12.5	7.5	1.7	10.2	7.2	1.4
250	12.4	11.8	1.05	–	–	–

Tab. II. Proline and hydroxyproline content in chemically non-modified collagen fibres from rat tail tendon in 12 days old and adult rats

Sample	% N	Amino acid g/100 g of protein		Amino acid g/100 g N of protein	
		Proline	Hydr-oxy-proline	Proline	Hydr-oxy-proline
Collagen from rat tail tendon 12 days old	17.0	18.6	7.6	11.0	5.7
Collagen from lungs of 12 days old rats isolated by LOWRY et al.[9]	14.2	18.4	6.9	7.8	5.0
Collagen from rat tail tendon 250 days old	–	18.8	10.9	–	–
Gelatine DGF	18.5	18.8	13.2	10.1	7.1

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proportion of soluble collagens in collagen fibre decreases during ageing[18]. This leads to the presumption of the splitting of a soluble collagen containing a higher concentration of hydroxyproline than the remaining insoluble collagen. HALL[19] actually reports that alkali soluble collagen from hide powder contains in a particular fraction an extremely high concentration of hydroxyproline (till 38 weight%). But this is in contradiction with the abovementioned analysis of soluble collagens[2-5].

To elucidate this discrepancy, we extracted all collagens from lung tissue using hot trichloracetic acid and the concentration of proline, and hydroxyproline was referred to the amount of nitrogen in extract. We found that, in lung extracts from various age groups of rats, the proline concentration does not change whereas the concentration of hydroxyproline is more than double during ageing: proline-hydroxyproline ratio amounts in collagens from 9 days old rats to 4.3; in 14 days old rats to 3.7; 30 days old rats to 3.0; 40 days old rats to 2.1; 60 days old rats to 1.6.

This increase in hydroxyproline concentration in collagen during ageing is not inherent to collagens extracted from organs only but to all forms of collagens in maturing rats. In Table II we present the results of analysis of chemically non-modified collagen fibers prepared mechanically from tail tendon of newborn and adult rats. In very young rats, the collagen fibre contains significantly less hydroxyproline than in collagen fibre from adult animals. The concentration of proline was the same in different samples, without regard to the age of the animals.

We suppose, therefore, that in early stages of development of rats the non-mature collagen is formed by a different aminoacid composition than is found in matured collagen. It is not yet clear how the maturation is realized, namely by which means the increase of hydroxyproline in collagen molecule occurs. It is worth mentioning that in peptides containing hydroxyproline isolated from different animal tissues[20], we found similar changes in the ratio of proline to hydroxyproline.

Zusammenfassung. Durch Bestimmung des Prolin- und Hydroxyprolingehaltes in unlösbarem Kollagen und in den gesamten Kollagenen der Lunge von Ratten verschiedenen Alters wurde bewiesen, dass in der frühen postnatalen Epoche der Ratte (bis zu 30 Tagen) die Menge des Hydroxyprolins im Kollagen eindeutig steigt, während sich die Menge des Prolins praktisch nicht ändert.

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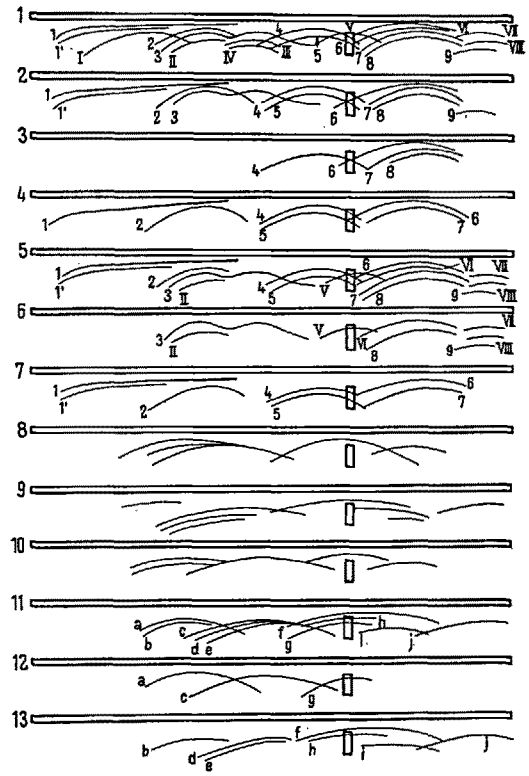
The Relation between some Serum and Liver Proteins in Rats Studied by Immunoelectrophoresis and by Ouchterlony's Double Diffusion Method

We investigated the relation between liver and serum proteins in rats both by immunoelectrophoresis[1,2] and by the double diffusion method in agar[3] with the technique of transferring agar discs with the isolated fractions[4].

The experiments were carried out with serum, liver homogenate and extracts from livers washed *in situ* from adult white rats. The extract marked R was obtained by centrifuging 30% of the homogenate of washed rat livers in a phosphate buffer (0.1 m pH 7.5) in a cooled laboratory centrifuge at 5000 g. Particles difficult to centrifuge were easily separated by the procedure quoted in one of our previous papers[5] after the addition of $^1/_3$ of the total volume of a rivanol (2-etoxy-6, 9-diaminoacridin lactate) solution. After centrifugation, the rivanol present in the supernatant was separated by absorption on active coal (Norit SX 30). The second extract analyzed, marked D, was obtained by centrifuging the 30% homogenate of washed rat livers with saline; particles difficult to centrifuge were precipitated with the help of dextran-sulphate in the presence of calcium chloride. The method was analogous to that used for the isolation of serum lipoproteins according to BURSTEIN[6]. Both extracts were dialyzed and lyophilized. All the work was performed at a temperature of 0–3°C.

The antisera used were obtained by immunizing 4 groups of rabbits for 5 weeks (each group consisting of 3–4 rabbits) with the following antigens: rat serum, 10% homogenate of washed rat livers in saline, extracts R and D (the solution of both extracts contained about 7% proteins).

The results of the immunoelectrophoretic analysis of the serum are given in the diagrams 1–4 (Fig.). On using specific antisera, we succeeded in detecting up to 17 precipitation lines. These lines were numbered with Arabic and Roman numerals. Arabic numerals were used



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