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The Amino Acid Composition of Human Collagens From Adult Dura Mater and Post-Menopausal Uterus

By J. J. HARDING

The British Gelatine and Glue Research Association, 2a Dalmeny Avenue, Holloway, London, N. 7

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The present results are an introduction to studies on the differences in amino acid composition between collagens from various normal and diseased human tissues. Adult dura mater represents a source of fairly pure collagen. The reasons for selecting uterus for study are: First, the shrinkage temperature and fluorescence of both pre- and post-menopausal uterine collagen have been shown (Brown, Consden & Glynn, 1958) to correspond to those of non-uterine collagen from a foetus or very young child. Secondly, uterus exhibits far greater synthesis and turnover of collagen than other tissues, and this process even continues into old age (Kao, Hilker & McGavack, 1961*a, b*). The third and major reason is interest in the rapid build-up during pregnancy and even more rapid resorption of uterine collagen during post-partum involution (Harkness & Harkness, 1954; Harkness & Moralee, 1956; Woessner, 1962; Morrione & Seifter, 1962).

Differences in the compositions of collagens from different tissues of one species have been established, e.g. the high hydroxylysine and low lysine contents of tendon collagen compared with skin and bone (Eastoe, 1955; Leach, 1957). However, collagens with greater metabolic activity, as in uterus, and those from rheumatic tissues have not yet been analysed completely for amino acids. Consden, Glynn & Stanier (1953) found no significant differences in the hydroxyproline and tyrosine contents of collagen extracted from rheumatic-fever nodules by autoclaving.

METHODS

Materials

Extractions were performed at room temperature unless otherwise stated. The tissues were extracted with approximately 20 times their wet weight of each solution.

Dura mater. A sample of dura mater from a 72-year-old woman was washed in water, freed from veins and extracted successively with (i) 0.9M-NaCl for 3 hr. (two changes) and (ii) citrate buffer, pH 3.7 (containing 0.1M-citric acid and 0.05M-sodium citrate), for 18 hr. The citrate buffer was shown by hydroxyproline determinations to have extracted less than 0.05% of the collagen. The extracted residue was dried under vacuum, giving material D₁. This contained impurities (see Results) and was therefore treated further with (a) trichloroethylene for 2 days, (b) ether-ethanol (3:1, v/v) for 2½ hr., (c) 3% (w/v) Na₂HPO₄ soln. for 2 days at 4°, and (d) saturated KCl soln. for 3 days at 4°. The residue was washed free of chloride and agitated vigorously twice with water and once with ethanol. It was then washed once in ethanol and twice in ether. The ether was decanted off and the fibres were spread in a flat glass dish and left overnight to equilibrate with atmospheric moisture. This material is D₂. The salt solutions extracted less than 0.05% by weight of the final residue, as shown by hydroxyproline determinations.

Uterus. The white outer layer of a uterus from a 59-year-old woman was removed and the remainder finely shredded in a food macerator and washed in water. It was then extracted with 0.9M-NaCl for 2 days, then ether-ethanol (3:1, v/v) for 2½ hr., 0.9M-NaCl for 3 hr., citrate buffer (pH 3.7) for 18 hr., saturated Na₂HPO₄ solution for 2 days at 4° and saturated KCl solution for 3 days at 4°. The residue was washed free of chloride and dried with ethanol and ether and equilibrated as for dura mater, giving the material U₁. This material contained non-collagenous proteins, probably elastin (see Results). Gelatin was extracted as follows: 0.28 g. of U₁ was suspended in 80 ml. of water. Sulphur dioxide was passed in until the pH fell to 2.5, and added for a further 2 hr. to maintain this pH. The beaker was then covered by polythene sheeting overnight (final pH 2.7). The supernatant was decanted and replaced by water. Sulphur dioxide was again passed in to lower the pH to 2.5. The beaker was then placed in a water bath at 80° for 2½ hr. After centrifuging, the residue was washed in water, ethanol and ether, equilibrated in air and weighed. The solution was warmed with about 0.2 g. of animal char-

coal to remove mucopolysaccharide, filtered, evaporated almost to dryness *in vacuo* over P_2O_5 and $CaCl_2$, and the last few millilitres were evaporated in a warm cupboard. The residue, U_2 , was left to equilibrate. All the salt and buffer extracts leading to U_1 were dialysed against water and centrifuged. The supernatant solutions contained less than 0.2% of the collagen. There was a small amount of fibrous material deposited from the KCl extract and a larger amount from both the NaCl extracts.

Moisture. The moisture content was determined by drying the finely divided material for 18 hr. at 105°.

Ash. The material was moistened with conc. H_2SO_4 , ignited on a platinum dish and heated at 550° for 4 hr., and the residue was weighed as ash.

Hydrolysis and analysis of collagen. A sample (0.15–0.20 g.) of each material (D_1 , D_2 , U_1 , U_2) was hydrolysed with 20 ml. of 20% (w/w) HCl at 100° in a sealed tube for 24 hr. The acid was removed by evaporation *in vacuo* over P_2O_5 and NaOH. The analyses were by the method of Moore & Stein (1951) as described by Eastoe (1955). The corrections described by Eastoe (1955) were applied to the values for glutamic acid, threonine, serine and amide nitrogen. Hydroxyproline was determined by the method of Neuman & Logan (1950) as modified by Leach (1960*a*). During amino acid analysis the eluate fractions from the chromatographic column were examined for fluorescence under an ultraviolet lamp (Hanovia, Fluorescence Model II) in a darkened room.

RESULTS

The amino acid contents of D_1 , D_2 , U_1 and U_2 are given in Table 1 as residues/1000 residues, which eliminates differences produced by non-protein

impurities or by slight errors in the standard amino acid solutions.

During hydrolysis of D_1 , some humin formation occurred and a globule of fat appeared on the surface of the liquid. This is evidence for mucoprotein and lipid impurities respectively. The presence of mucoprotein was also indicated by the amino acid compositions. The amino acids that are higher in D_1 than in D_2 correspond to those present in large amounts in the mucoproteins associated with collagen (Leach, 1960*b*). For these reasons D_1 was purified further. If glycine, proline and hydroxyproline are taken as the typical collagen amino acids, the results (cf. D_1 and D_2 in Table 1) suggest that about 3% of protein impurity had been removed. The extent of removal of all impurities is shown by the increase of hydroxyproline from 12.6 g./100 g. in D_1 to 13.2 g./100 g. in D_2 . Since the amide-nitrogen content of D_2 was not significantly less than that of D_1 , the treatment to which the latter had been subjected was not too severe.

An unknown component of U_1 was detected as a large ninhydrin-positive peak immediately following cysteic acid on the 100 cm. column of Moore & Stein (1951). The fractions containing this component were yellow and fluorescent under ultraviolet light. This component was lost on gelatinization and was not found in a second post-menopausal uterus. It therefore appears to be an artificial component, perhaps arising from one of

Table 1. *Amino acid compositions of uterus, dura mater, bone and tendon collagens, reticulin and elastin*

The compositions are expressed as residues/1000 residues. Figures for bone and tendon were taken from Eastoe (1955), those for reticulin from Windrum *et al.* (1955) and those for elastin from Partridge & Davis (1955).

	Dura mater		Uterus		Bone	Tendon	Kidney reticulin	Elastin
	D_1	D_2	U_1	U_2				
Ala	106.7	106	103.2	95.9	113.5	110.7	96.5	218
Gly	321	327	301.8	337.1	319	324	309	312
Val	27.6	26.2	36.5	21.6	23.6	25.4	26.8	129
Leu	27.6	27.1	37.5	24.5	25.5	26.0	35.8	59.6
Ileu	12.9	10.7	17.4	10.8	13.3	11.1	18.0	25.0
Pro	120.4	125	109.6	108.2	123.4	126.4	97.2	99.7
Phe	15.7	14.7	18.5	12.3	13.9	14.2	18.1	32.8
Tyr	4.7	4.0	10.4	2.2	4.5	3.6	3.0	6.5
Ser	38.0	31.1	40.2	41.9	35.9	36.9	42.8	8.5
Thr	19.0	18.3	22.7	20.6	18.4	18.5	21.9	8.8
Cysteic acid	Trace	Trace	2.8	Trace	Trace	Trace	—	1.3
Met	6.6	3.7	7.2	5.8	5.3	5.7	8.6	Trace
Arg	45.9	46.5	43.7	49.0	47.1	49.0	45.3	62.7
His	5.1	5.1	7.3	5.4	5.8	5.4	5.3	0.3
Lys	24.6	25.0	28.1	24.9	28.0	21.6	21.6	3.1
Asp	49.2	48.9	53.7	52.5	47.0	48.4	52.9	7.2
Glu	71.7	71.6	75.8	73.6	72.2	72.3	76.7	14.8
Hypro	99.1	102	80.4	108.6	100.2	92.1	107.7	10.7
Hylys	3.6	4.3	3.2	5.1	3.5	8.9	12.2	—
Amide N	40.5	39.0	45.8	41.9	37.3	44.0	43.0	—
Total hydroxyamino acids	164.4	159.7	—	178.4	162.5	160.0	187.6	—

the numerous drugs prescribed for the woman, and has been omitted from the calculations in Table 1. It was present in large quantity, being approximately 5% of U_1 , assuming a mol.wt. of 200 and colour factor of 1.00. The high values for valine, leucine and isoleucine in U_1 indicate the presence of a large amount of elastin, an analysis of which (Partridge & Davis, 1955) has been included in Table 1 for comparison. Gelatinization separated the collagenous material from the elastin, which remained as a residue (13% by weight). U_2 then shows an amino acid composition typical of collagen.

Removal of elastin is indicated by a decrease in the content of alanine, valine, leucine and isoleucine. An increase in the proportion of collagen content is shown by an increase in the glycine, hydroxyproline and hydroxylysine.

DISCUSSION

These results confirm that dura mater consists largely of collagen with a typical amino acid composition. A previous analysis of dura mater (Wunderly & Wuhrmann, 1954) was incomplete, hydroxyproline, hydroxylysine and histidine having been omitted. Thus adequate comparison with the present results is not possible.

The results for U_1 and U_2 indicate that the residue remaining after prolonged extraction of uterus with salt and buffer solutions consists of about 80% of collagen, the remainder being mainly elastin. These two components were separated by conversion of the collagen into gelatin. This procedure has been shown (Eastoe, 1955) to result only in small changes of composition of gelatins extracted into acid media. Thus the analysis of U_2 may be taken as that of uterine collagen.

The patterns of amino acid composition of collagens from dura mater and post-menopausal uterus are generally similar to those of collagen from bone and tendon (Eastoe, 1955), which have been included in Table 1. The value for serine in dura mater is lower than that in bone and tendon, but the total hydroxyamino acid content is the same for the three collagens. Uterine collagen shows certain differences, particularly in the high content of the hydroxyamino acids serine, threonine and hydroxyproline. In this respect U_2 resembles the protein of reticulin (Windrum, Kent & Eastoe, 1955), the analysis of which has been included in Table 1. Also, like reticulin, U_2 has a low proline and high aspartic acid content. Citrate buffer extracted a negligible amount of collagen from uterus, despite the apparent physical immaturity (Brown *et al.* 1958). Woessner (1962) found that even in post-partum rat uterus only

2.5% of the collagen is extractable by aqueous sodium chloride and 0.5M-acetic acid.

The present results and those of Eastoe (1955) provide a suitable range of human collagens with which to compare collagens from uterus of other ages and at different stages of pregnancy and also collagens from rheumatoid tissue.

SUMMARY

1. The amino acid compositions of collagens from human dura mater and human post-menopausal uterus have been determined and found to be similar to those of bone and tendon collagen. The uterine collagen closely resembles the protein fraction of reticulin in composition.

2. After extraction with sodium chloride solution no collagen was extracted by citrate buffer, pH 3.7.

3. The hydroxylysine and lysine contents of both uterus and dura-mater collagens are intermediate between those of bone and tendon collagens.

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