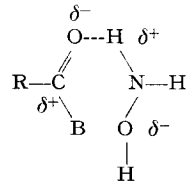
to decompose in aqueous or alcoholic solution. It appears that the nucleophilic attack by the hydroxylamine oxygen atom, which may be expected to carry a partial negative charge because of the high electronegativity of oxygen, is aided by an increase in the polarization of the carbonyl group induced by simultaneous electrophilic attack by the hydroxylamine amino group (*cf.* [8]).



Alternatively, the reactive species of hydroxylamine in solution may be the zwitterion $H_3\overset{+}{N}-O^-$, although spectroscopic evidence has been interpreted to indicate that this form is not present in the hydroxylamine crystal[9].

The occurrence of this reaction provides experimental support for the concept that the hydroxyl group of serine may react to form O-acyl serine as an intermediate in the hydrolysis and transfer of activated acyl groups by chymotrypsin and similar enzymes[10], provided that the serine reactivity is enhanced by neighboring electrophilic groups on the enzyme.

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Converse Memorial Laboratory, Harvard University, Cambridge, Mass. (U.S.A.) WILLIAM P. JENCKS*

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* U.S. Public Health Service Fellow. Present address: Graduate Department of Biochemistry, Brandeis University, Waltham 58, Mass. (U.S.A.).

The amino acid composition of the collagen fractions of rabbit skin

Three collagen fractions are now recognized: neutral salt-soluble collagen[1,2], citrate-soluble collagen[3] and insoluble collagen which constitutes the bulk of the collagen in connective tissue and can be rendered soluble by transformation into gelatin.

A number of complete amino acid analyses of mammalian insoluble collagen and of gelatin have been made[4,5], and BOWES *et al.*[4] have analysed citrate-soluble collagen from calf-skin. However no analysis is available for neutral salt-soluble collagen nor have analyses been made of all three types of collagen from the same source. This paper reports a method for the isolation and purification of neutral salt-soluble collagen and the amino acid analysis of all three types of collagen isolated from the skins of young growing rabbits.

All extractions were carried out at 2° in the presence of *sec.*-octanol as preservative. Neutral salt-soluble collagen as prepared by HIGHBERGER *et al.*[7] is soluble only with considerable difficulty after purification and may become completely insoluble[6]. It was therefore prepared by the following method, some of the physical properties of this fraction having already been described[1].

The skins were extracted several times with 0.2*M* NaCl, pH 7.4, for 24 h and the extracts pooled and filtered after centrifuging for 30 min at 12,000 *g*. NaCl to a final concentration of 20% (w/v) was added and the precipitate removed by centrifuging at 12,000 *g* for 30 min. The precipitate was redissolved in 0.2 *M* NaCl, pH 7.4, and the insoluble residue removed by centrifuging at 12,000 *g*. An equal volume of 5 *M* NaCl was added and the precipitate removed by centrifugation, redissolved in 0.2 *M* NaCl and this step repeated. The final solution in 0.2 *M* NaCl was faintly opalescent, and was clarified by centrifuging at 100,000 *g* for 1 h. 5 *M* NaCl was added

TABLE I

THE AMINO ACID COMPOSITION OF CITRATE-SOLUBLE, NEUTRAL-SOLUBLE, AND INSOLUBLE COLLAGENS FROM RABBIT SKIN AND COMMERCIAL ACID PROCESS RABBIT GELATIN

	g Amino acid/100 g dry ash-free protein				*Nitrogen as % of total protein nitrogen*			
	citrate-soluble	*neutral salt-soluble*	*insoluble*	*commercial gelatin*	*citrate-soluble*	*neutral salt-soluble*	*insoluble*	*commercial gelatin*
Alanine	10.9	9.1	8.6	9.9	9.5	8.6	8.2	8.5
Glycine	26.0	22.5	21.9	25.8	27.1	25.0	24.8	26.4
Valine	3.24	3.20	2.40	2.56	2.16	2.28	1.74	1.68
Leucine	3.65	3.95	2.89	3.07	2.17	2.52	1.87	1.80
Isoleucine	1.60	2.26	1.50	1.82	0.95	1.43	0.92	1.07
Proline	15.4	13.0	15.5	16.0	10.5	9.42	11.4	10.7
Phenylalanine	2.29	2.53	1.93	2.44	1.09	1.27	0.90	1.03
Tyrosine	0.38	1.03	0.34	0.58	0.16	0.48	0.16	0.24
Serine*	4.43	4.73	4.00	3.85	3.30	3.76	3.21	2.81
Threonine*	2.71	3.31	2.23	2.52	1.78	2.32	1.59	1.63
Cystine**	trace	trace	0.05	0.13	trace	trace	0.04	0.08
Methionine***	0.75	0.98	1.23	0.85	0.39	0.54	0.70	0.44
Arginine	9.2	8.4	7.5	8.7	16.6	16.1	14.6	15.3
Histidine	0.75	1.13	0.82	0.87	1.15	1.83	1.35	1.29
Lysine	4.55	4.73§	3.79	4.23	4.87	5.40	4.40	4.45
Aspartic acid	6.9	7.4	6.3	6.7	4.03	4.64	4.03	3.88
Glutamic acid	11.3	11.3	9.6	10.5	6.0	6.4	5.5	5.5
Hydroxyproline	14.4	12.9	12.9	14.5	8.7	8.2	8.3	8.5
Hydroxylysine	0.83	0.79	0.77	0.75	0.80	0.78	0.81	0.71
Total	119.3	113.2	104.2	115.8				
Amide N	0.62	0.72	0.66	0.65	3.46	4.26	3.97	3.60
Av. residue weight	91.4	93.6	92.4	91.8				
Z (CHIBNALL, 1942)[10]	119.7	119.2	119.5	119.6				
% wt. recovery	99.7	95.0	87.2	96.9				
% N recovery					104.7	105.2	98.5	99.6
% Ash	0.7	0.9	12.0	1.3				
% Moisture	22.1	22.0	17.0	15.2				
% N					17.9	16.8	16.5	18.1

* Corrected for decomposition during hydrolysis.
** Present in hydrolysate as cysteic acid.
*** Including methionine sulphoxide calculated as methionine.
§ 0.43% ornithine was found in an experiment using Zeocarb 225. This peak is overlapped by lysine on the Dowex-50 column.

to the clear solution thus obtained until a faint opalescence developed and allowed to stand for several hours at 2° when a gelatinous precipitate was formed. This precipitate was removed by centrifugation and dissolved in 0.01*M* acetic acid and after prolonged dialysis against 0.01*M* acetic acid the solution was freeze-dried. At pH 7 the protein gave a single hypersharp peak both in the ultracentrifuge and on electrophoresis.

Citrate-soluble collagen and gelatin from the residual insoluble collagen were prepared as described previously[8].

The analyses (Table I) were carried out on columns of Dowex-50 (nominal 12% cross linking) by the method of MOORE AND STEIN[9] as modified by EASTOE[5]. These results were confirmed by using a single column of Zeocarb 225[11], which in addition showed ornithine to be present in the neutral salt-soluble collagen. Since the nitrogen content of the gelatin from the young rabbits was low, a further analysis of a commercial rabbit gelatin was carried out.

The results showed that the amino acid compositions of the three fractions were essentially the same although there were some differences, notably the lower value for proline and hydroxyproline and the higher value for tyrosine found for neutral salt-soluble collagen. These differences may be accounted for by the presence of a non-collagenous protein in the neutral salt-soluble fraction. On this assumption this fraction as isolated by the method described is 94% pure, assuming the value 8.3% hydroxyproline (amino acid N % total N) obtained for citrate-soluble

collagen to be the true value for pure collagen. The differences shown here between neutral salt-soluble and citrate-soluble collagen affect the same amino acids as the differences described by BOWES *et al.*[4] between citrate-soluble and insoluble collagen from bovine skin. These differences were attributed by these authors to the presence of a mucoprotein containing hexosamine in insoluble collagen. However, hexosamine is absent from neutral salt-soluble collagen so that this suggestion would not apply here.

The presence of ornithine in collagen has not previously been reported but its origin is not known.

Sear Surgical Laboratory, The Boston City Hospital, Boston, Mass. (U.S.A.) D. S. JACKSON
The British Gelatine and Glue Research Association, 2a Dalmeny Avenue, London, (England) A. A. LEACH
The National Institute for Medical Research, London, (England) S. JACOBS

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Fractionation of ribonucleic acid by precipitation with neutral salts*

Methods of fractionation of ribonucleic acid (RNA) preparations have been based on fractional precipitation or extraction by suitable solvents[1,2], elution from ion-exchangers[3,4], or dissociation of protamine nucleate[5]. The last method is used for obtaining fractions with different base composition, while the others give fractions with different molecular weight.

High-molecular-weight RNA has been shown to be precipitated from neutral aqueous solution by high concentrations of salts such as NaCl or $(NH_4)_2SO_4$[6,7], or by relatively low concentrations of $MgCl_2$ or $CaCl_2$[8]. We have now been able to divide yeast RNA into fractions with different base composition by the fractional precipitation with neutral salts. This method has the advantages of being simple and being applicable to high-molecular-weight RNA preparations and to any size of sample.

RNA was prepared from baker's yeast by the method of CRESTFIELD, *et al*[6]. It was dissolved in dilute saline, and then an appropriate volume of the concentrated salt solution (NaCl or $MgCl_2$) was added. The mixture was kept at 0–5° for 15–20 h. The precipitate formed was separated by centrifugation. In this way the sample may be fractionated successively into several fractions. RNA's in the precipitate and in the supernatant were analyzed for their base composition[3] after being recovered by ethanol. The results are shown in Table I.

It can be seen from Table I that the fractions thus obtained, except those of Expt. 4, differ from each other with respect to their guanine and cystoine contents; the fraction more soluble in salt solution tends to be richer in guanine and cytosine than the one less soluble. Especially the fraction soluble in 2 *M* NaCl obtained in Expt. 2 had markedly high guanine and cytosine contents. On the other hand, the composition of the readily-precipitable fraction did not vary greatly from the original sample. In Expt. 4, the readily-precipitable fraction was sub-divided into smaller fractions, but no significant variation of the composition could be observed, although it may also be possible that $MgCl_2$ is less effective for fractionation than NaCl, since even the supernatant fraction in this experiment differed little from the starting material.

From these results it seems possible that there are some RNA molecules which are exceptionally rich in guanine and/or cytosine in yeast RNA preparations obtained by the method of CRESTFIELD *et al.*[6]; they may represent a relatively small fraction and can be concentrated in a fraction

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