- Hunter, F. E., Davis, J. & Carlat, L. (1956). *Biochim. biophys. Acta*, **20**, 237.
- Kielley, W. W. (1951). *Phosphorous Metabolism*, vol. 1, p. 516. Ed. by McElroy, W. D. & Glass, B. Baltimore: The Johns Hopkins Press.
- Kielley, W. W. & Kielley, R. K. (1951). *J. biol. Chem.* **191**, 485.
- Kunitz, M. & McDonald, M. R. (1946). *J. gen. Physiol.* **29**, 393.
- Lardy, H. A. & Wellman, H. (1953). *J. biol. Chem.* **201**, 357.
- Mager, J. & Avi-Dor, Y. (1956). *Arch. Biochem. Biophys.* **62**, 40.
- Mager, J. & Blank, I. (1954). *Nature, Lond.*, **173**, 126.
- Martel, A. E. & Schwarzenbach, G. (1956). *Helv. chim. acta*, **39**, 653.
- Peters, R. A. & Hall, R. J. (1957). *Biochim. biophys. Acta*, **26**, 433.
- Schneider, W. C. (1948). *J. biol. Chem.* **176**, 259.
- Swanson, M. A. (1956). *Biochim. biophys. Acta*, **20**, 85.
- Tapley, D. F. (1956). *J. biol. Chem.* **222**, 325.

Studies of Invertebrate Collagen Preparations

By M. R. WATSON AND N. R. SILVESTER

Medical Research Council, Biophysics Research Unit and Wheatstone Physics Laboratory, King's College, London, W.C. 2

(Received 4 June 1958)

The collagens may be defined as a group of fibrous proteins which give a characteristic wide-angle X-ray-diffraction pattern (Astbury, 1938; Bear, 1952). Although the collagens are widely distributed throughout the animal kingdom (Bear, 1952; Rudall, 1955), physical and chemical studies have been largely confined to those of vertebrates and particularly of mammals, because of their industrial and clinical importance. The vertebrate collagens form a compact group in their amino acid composition (Eastoe & Leach, 1958), and it is of interest to extend these studies to collagens from a wider range of animals, since one might expect that the greater the range of collagens for which data are available the greater may be the chance of detecting correlations between the chemical and physical properties of these proteins. It was with this aim in view that the cuticle of the earthworm was studied by Watson (1958*a*). The present publication is concerned with studies on two other collagens, those from the cuticle of *Ascaris lumbricoides* and from the Cuvierian tubules of *Holothuria forskali*.

Ascaris cuticles were first shown by Fauré-Fremiet & Garrault (1944) to give the characteristic wide-angle X-ray-diffraction pattern of collagen. For many years previously, however, the cuticle had been studied by chemical and histological methods. It is a very complex structure, and Chitwood & Chitwood (1937) have distinguished nine morphologically distinct layers. Chitwood (1936) also describes the methods which may be used to separate the various substances in the cuticle and discusses their morphological location. Bird (1956, 1957) has examined the amino acid composition of the whole cuticle by

paper chromatography, and also of extracts prepared from it by methods based on those of Chitwood (1936), and has reported a quantitative amino acid analysis in which values are given for most of the amino acids present.

The ejected filaments of the sea cucumbers were shown to contain collagen by Astbury (1939) from the X-ray-diffraction evidence. These filaments arise by extension from the Cuvierian tubules of the animals, of which the present example is *Holothuria forskali*.

An analytical investigation of the whole tissues preceded attempts to isolate and analyse the collagen proteins, for two reasons. First, such a control is useful in deciding whether the composition of the purified collagen is representative of the bulk of the collagen present, and in estimating the yield of collagen in the purification process. Secondly, it helps to determine which amino acid values in the final analysis are apt to be greatly influenced by the presence of residual impurities. Some of the preliminary results with the filaments of *Holothuria forskali* have been reported (Watson, 1958*b*).

EXPERIMENTAL

Preparation of materials for analysis

Cuvierian tubules of Holothuria forskali. Tubules were collected from the living animals by lifting them out of the sea water in which they had been transported to the laboratory. In a few moments the tubules were extruded through the cloaca as long, intensely sticky filaments. These were washed in several changes of water, two changes of ethanol and two changes of ether, and allowed to dry in air. About 2 g. of air-dried filaments was obtained from three animals. Two such preparations were made.

Table 1. *Ash, total nitrogen, sugars and physical properties*

Values are given as g./100 g. of dry ash-free specimen.

	Cuvierian tubules of <i>Holothuria forskali</i>			Cuticle of <i>Ascaris lumbricoides</i>		
	Whole tubules		Collagen fibres	Whole cuticle		Gelatin
	Prep. 1	Prep. 2		Prep. 1	Prep. 2	
Ash	3.01	2.69	0.30	0.28	0.26	0.10
Total N	15.6	16.1	17.0	16.7	16.6	17.3
Hexose	8.2	7.8	4.5	0.19	0.19	0.50
Pentose	0.46	0.40	0.29	0.10	0.19	0.15
Hexosamine	1.34	1.34	0.95	0.79	0.51	1.29
Shrinkage temp.	55°	59°	59°	59°	59°	—
640 Å periodicities	Present*			Absent†		

* See Plate 5.

† Picken, Pryor & Swann (1947) and Bird & Deutsch (1957).

Collagen fibres were isolated from the extended tubules by the following procedure. Air-dried filaments from the second preparation (560 mg.; 10.5% of moisture) were incubated with 0.1% of trypsin in tap water at 37° for 24 hr. under toluene. The tubules were removed from the digest and broken up by homogenization in 100 ml. of tap water for 5 min. at low speed in a Waring Blender. The fibres were collected by centrifuging at 1000 g for 0.5 hr., after which they were washed twice in water and allowed to stand in N-HCl at 4° for 40 hr. In contrast with the behaviour of vertebrate collagens generally, no swelling occurred in the acid. The acid was then removed by washing with water, the fibres were rinsed in two changes of ethanol and two changes of ether, and allowed to dry in air. The yield of collagen fibres was 325 mg. air-dried wt. (moisture 9.6%). Thus the isolated collagen fibres amounted to some 60% of the dry wt. of the whole tubules, and the recovery of collagen (based on the recovery of hydroxyproline) was about 75%.

Cuticles of Ascaris lumbricoides. Two dozen *Ascaris* were dissected and the internal organs removed. The muscle layers of the body wall were scraped off under water with the blade of a scalpel. The cuticles were thoroughly rinsed in water and kept under water for 16 hr. at 4°. The cuticles were given a final rinse in water, washed in two changes of ethanol and two changes of ether and allowed to dry in air. About 800 mg. of air-dried cuticles was obtained.

The extraction of a collagen-rich fraction from a further preparation of the cuticles was attempted by the following method. Cuticles (air-dried wt. 1003 mg.; moisture 14.1%) were extracted with 150 ml. of water at 37° for 3 days under toluene. Only 2.48 mg. of N, equivalent to 1.7% of the total cuticular N, passed into solution. Thus either the water-soluble fraction of the cuticle described by Chitwood (1936) and Bird (1957) must have been extracted during the preparation of the cuticles, or its properties were changed by the drying procedure. The cuticles were then digested with trypsin (0.1% in 100 ml. of tap water at 37° for 24 hr. under toluene) and washed with water to remove the enzyme and soluble degradation products; finally the collagen was brought into solution by autoclaving the cuticles in 150 ml. of water at 135° for 2.5 hr. The air-dried residue weighed 94 mg. The gelatin was recovered from solution by evaporation on a steam bath in an air blast, and weighed 500 mg. (air-dried wt.; moisture 14.3%). Thus the

gelatin amounted to 50% of the weight of the whole cuticles. The recovery of collagen (based on the recovery of hydroxyproline) was about 57%. The gelatin solution did not gel at 16° after evaporation to a concentration of about 10%.

Analytical

Moisture and total nitrogen. The determinations were performed according to Chibnall, Rees & Williams (1943).

Amino acid analyses. These were carried out by the method of Moore & Stein (1951). The corrections were the same as for earthworm cuticle (Watson, 1958a). It was not necessary to use the special method for proline described in the last-named paper, since the samples contained enough of the imino acid to allow the estimation to be performed in the usual way.

Amide nitrogen. The method of Bailey (1937) was used.

Estimation of sugars. This followed the same standard colorimetric methods as were used for earthworm cuticle (Watson, 1958a), except that the anthrone reaction for hexoses was carried out by the modification described by Windrum, Kent & Eastoe (1955). Hexose is expressed as galactose, pentose as arabinose and hexosamine as glucosamine.

RESULTS

The two preparations of *Ascaris* cuticles gave analytical results which were in fairly good agreement (Tables 1 and 2). The material contained little ash. The low recovery of weight in the amino acid analysis suggests that 10% of other substances with low nitrogen contents may be present in the cuticle. On the basis of a positive Molisch reaction previous workers have concluded that polysaccharides are present, but the analyses listed in Table 1 show only a small amount of hexosamine. The value for hexoses is of the order expected for the colour contribution of the amino acid residues to the anthrone reaction (Moss, 1955). The absence of large amounts of polysaccharide in the preparations is also suggested by the following observations. Little humin appeared upon hydrolysis for the determination of the amino acids, and no red

Table 2. *Amino acid analyses*

Values are expressed as g. of amino acid/100 g. dry ash-free sample.

	Cuvierian tubules of <i>Holothuria forskali</i>			Cuticle of <i>Ascaris lumbricoides</i>		
	Whole tubules		Purified collagen	Whole cuticles		Gelatin
	Prep. 1	Prep. 2		Prep. 1	Prep. 2	
Alanine	7.4	8.2	9.1	6.3	6.3	5.7
Glycine	17.2	18.2	20.8	18.9	18.5	19.8
Valine	3.17	3.17	2.24	1.76	1.78	1.37
Leucine	3.60	3.74	3.25	2.53	2.32	2.22
Isoleucine	2.33	2.22	1.56	2.02	1.89	1.67
Proline	6.7	7.1	8.4	30.9	31.2	29.7
Phenylalanine	1.96	2.22	1.60	1.34	1.29	1.11
Tyrosine	1.82	2.24	1.85	0.40	0.62	0.20
Serine	5.4	5.4	5.2	2.57	2.57	2.20
Threonine	6.3	6.3	5.5	2.23	2.10	1.98
Cystine	0.14	—	—	*	*	*
Methionine	0.97	1.06	0.93	1.34	1.40	1.66
Arginine	7.3	7.3	7.3	6.5	6.4	6.8
Lysine	2.62	2.47	1.62	5.4	5.3	5.0
Histidine	1.72	2.14†	0.68	1.32	1.25	1.19
Hydroxylysine	0.42	—	0.69	0.0	0.0	0.0
Aspartic acid	8.4	9.4	9.1	8.5	8.7	8.6
Glutamic acid	11.8	13.1	13.3	10.7	10.5	10.4
Hydroxyproline	4.4	5.0	6.4	2.53	2.44	2.84
Amide N	0.968	0.962	1.06	0.569	0.559	0.571
Mean residue wt.	95.2	96.1	92.7	93.7	93.8	93.3
Recovery of N (%)†	96.3	98.2	93.0	96.0	96.2	92.0
Recovery (by wt. %)§	87.6	92.4	88.2	89.2	88.6	87.7
* Present, not determined (see text).						
† Including hexosamine.						
‡ Including hydroxylysine.						
§ Including sugar residues.						

Table 3. *Comparison of the mean of the present values for the amino acid composition of Ascaris cuticles with the literature values*

Values are expressed as g. of amino acid nitrogen/100 g. of total nitrogen.

Method of analysis ...	Moore & Stein (1950)	Moore & Stein (1951)
Hydrolysis ...	80 % formic acid-11.7N-HCl (1:1)	6N-HCl at 110° for 24 hr.
Reference ...	Bird (1957)	Present study
Alanine	5.6	6.0
Glycine	18.1	20.4
Valine	4.25	1.29
Leucine	1.94	1.55
Isoleucine	1.44	1.25
Proline	22.1	22.7
Phenylalanine	0.88	0.67
Tyrosine	0.50	0.24
Serine*	1.78	1.82
Threonine*	1.44	1.47
Methionine	1.00	0.77
Arginine	11.2	12.5
Histidine	1.94	2.09
Lysine	7.7	6.2
Aspartic acid	5.2	5.4
Glutamic acid	5.9	6.0
Hydroxyproline	—	1.58
Hydroxylysine	—	0.0
Amide N	—	3.39
Unknown	1.51	—
Total	92.5	95.3

* Not corrected for decomposition during hydrolysis.

peak at about tube 50 of the amino acid separation was seen (Dustin, Czajkowska, Moore & Bigwood, 1953). The two preparations gave different analyses for total hexosamines, although two independent determinations on each preparation were in excellent agreement. A small amount of glucosamine was observed on the short column of the amino acid separation (Eastoe, 1954) when large loads (20 mg. or more) of hydrolysate were used. The amount recovered in this way was estimated as 0.28 % for each preparation.

The presence of lipids in the cuticle has been suggested by many authors, but little quantitative information has appeared. It has been found that refluxing the cuticles with ethanol-ether (1:1) for 8 hr. removes only 0.3 % of their weight, and that most of this is a red pigment which has not been examined further.

The amino acid analyses which we have obtained for the whole cuticle (mean of the two preparations) are compared in Table 3 with the analysis calculated from the data reported by Bird (1957). One large discrepancy is found for valine.

The analytical results for the Cuvierian tubules of *Holothuria forskali* are also listed in Tables 1 and 2. The total hexosamine contents of the two preparations were identical at 1.34 %. Both glucosamine and galactosamine were identified on the short column of the amino acid separations (Eastoe, 1954), the amounts recovered in this way

being 0.10 and 0.41 % respectively for preparation 1 and 0.14 and 0.33 % for preparation 2.

The tubules were first reported to give the collagen type of wide-angle X-ray-diffraction pattern by Astbury (1939), and we have confirmed this observation (Plate 5a). Contrary to previous belief (Bear, 1952), the fibrils teased out from the tubules give evidence of large-scale periodicities

Fig. 1. Relative structure amplitudes, $|F_k|$, corresponding to the low-angle X-ray-diffraction pattern in Plate 5b (thick lines), are compared with those obtained by Tomlin & Worthington (1956) for ox Achilles tendon stained with phosphotungstic acid. The $|F_k|$ for the tubules were obtained from integrated layer-line intensities measured with a recording microdensitometer (Walker, 1955). k represents layer-line index.

Fig. 2. Infrared spectra of collagen in the 3μ region. (a) Spectrum of earthworm cuticle recorded at a relative humidity of 60%, showing absorption at 3330 cm^{-1} due to the N-H stretching vibration. (b) Spectrum of the same sample partially deuterated after exposure for 1 hr. to D_2O vapour. The other two invertebrate collagens, and also vertebrate collagens, have yielded spectra virtually identical with those illustrated.

when examined in the electron microscope (Plate 5c). Also low-angle X-ray-diffraction photographs have been obtained for the stretched whole filaments, stained with phosphotungstic acid, which show a number of meridional reflexions corresponding to an axial repeat of about $650\text{ \AA}$ (Plate 5b). The relative structure amplitudes of the various layer lines in Plate 5b are compared in Fig. 1 with values taken from a low-angle X-ray-diffraction study by Tomlin & Worthington (1956) of ox Achilles tendon stained with phosphotungstic acid. Since it was not possible to obtain an accurate value of the integrated intensity at the first-layer line in the photograph from the Cuvierian tubules, the sets of values in Fig. 1 are normalized to show the same relative value of $|F_k|$ at $k=2$. The main features shown by the figure are the strong fourth- and sixth-layer lines and an abnormally depressed ninth; the tenth and eleventh lines show moderate expression. Marks, Bear & Blake (1949) give rather similar descriptions of photographs from three other echinoderm sources.

Mr G. R. Wilkinson of this Laboratory has kindly examined the infrared absorption spectra of the collagens studied here and also of earthworm cuticle (Watson, 1958a). All three materials show a characteristic absorption band at 3330 cm^{-1} , which has been found previously among the proteins only in the vertebrate collagens, and have a slow rate of deuteration. Non-collagenous proteins have absorption maxima in the range $2990\text{--}3010\text{ cm}^{-1}$. Typical spectra are shown in Fig. 2.

DISCUSSION

The amino acid analyses for the collagens studied here, together with the analyses for earthworm cuticle (Watson, 1958a), are presented in Table 4 in a form especially suitable for structural studies, namely the number of residues of each amino acid which occur on the average in every 100 residues. A survey of the amino acid compositions of the vertebrate collagens in these units has been presented by Eastoe & Leach (1958). In Table 4 analyses for the whole tissues are included with those of the purified gelatins or collagen therefrom. It is not claimed that the methods of purification which have been used are necessarily the best possible, and the inclusion of values for the whole tissues may enable future workers to compare the efficacy of various methods. This is especially the case with the cuticles of *Lumbricus* and *Ascaris*, where the whole tissues show a reasonable constancy of composition, when the methods described for their preparations are closely followed. Two preparations of the Cuvierian tubules had rather different compositions, particularly in the contents of hydroxyproline and the acidic amino acids.

Average values are listed in Table 4. The shrinkage temperatures of the two preparations also differed by about 4° (Table 1).

In all experiments the purification of the collagen protein has resulted in increases in the proportions of glycine and hydroxyproline, as might have been expected. It is difficult to decide whether the

glycine contents of the proteins are slightly less than those commonly found in the vertebrate collagens, or whether this means that our preparations contain more non-collagenous proteins. Considering the extensive range of composition which is shown, the glycine contents are remarkably constant.

Table 4. *Proportions of the amino acid residues in three invertebrate tissues, and in collagen and gelatin preparations obtained therefrom*

	Earthworm (<i>Lumbricus</i>) cuticles (Watson, 1958a)	Earthworm (<i>Lumbricus</i>) cuticle gelatin (Watson, 1958a)	Whole Cuvierian tubules of <i>H. forskali</i>
Glycine	30.2	32.4	27.7
Alanine	10.0	10.3	10.3
Valine	2.13	1.71	3.18
Leucine	3.30	2.85	3.28
Isoleucine	1.71	1.53	2.04
Phenylalanine	1.34	0.52	1.48
Proline	1.42	1.31	7.0
Total non-polar	50.1	50.6	55.0
Hydroxyproline	15.5	16.5	4.2
Serine	10.3	10.5	6.0
Threonine	6.0	5.2	6.2
Total hydroxylic	31.8	32.2	16.4
Free carboxylic acids	4.07	4.00	9.5
Amide	9.9	9.7	8.2
Arginine	2.13	2.11	4.92
Lysine	1.54	1.47	2.04
Hydroxylysine	0.0	0.0	0.36
Histidine	0.06	0.0	1.46
Total basic	3.73	3.58	8.8
Tyrosine	0.40	0.0	1.31
Methionine	0.0	0.0	0.80
	Collagen fibres from the Cuvierian tubules of <i>Holothuria forskali</i>	Whole <i>Ascaris</i> cuticle	Gelatin from <i>Ascaris</i> cuticles
Glycine	30.8	26.5	28.6
Alanine	11.4	7.6	6.9
Valine	2.13	1.61	1.26
Leucine	2.76	1.97	1.84
Isoleucine	1.32	1.59	1.38
Phenylalanine	1.08	0.84	0.73
Proline	8.1	28.8	28.0
Total non-polar	57.6	68.9	68.7
Hydroxyproline	5.4	2.00	2.35
Serine	5.5	2.61	2.27
Threonine	5.1	1.93	1.80
Total hydroxylic	16.0	6.5	6.4
Free carboxylic acids	9.3	10.3	10.3
Amide	8.4	4.29	4.43
Arginine	4.63	3.95	4.24
Lysine	1.23	3.91	3.71
Hydroxylysine	0.47	0.0	0.0
Histidine	0.48	0.89	0.84
Total basic	6.8	8.8	8.8
Tyrosine	1.14	0.29	0.12
Methionine	0.69	0.98	1.21

It would seem from the data that the amino acid compositions of the purified collagens and gelatins are representative of the collagen which forms the bulk of the samples. The only large changes which have taken place upon purification are the fall in the values for histidine and lysine in the collagen from the Cuvierian tubules, which is probably due to the removal of non-collagenous proteins. In this particular purification the value for tyrosine did not fall as much as was expected, and it is probable that this collagen contains a higher proportion of these residues than do most other collagens.

A consideration of the data in the light of the extensive analyses of the vertebrate collagens reviewed by Eastoe & Leach (1958) has yielded the results that are listed in Table 5. First, this table indicates the features shared by the collagens for which reasonably complete amino acid analyses are now available, and which serve to distinguish them from all other proteins. Among the common physical features is the wide-angle X-ray-diffraction pattern (often adopted as the definition of collagen) and the infrared-absorption band at 3330 cm.⁻¹. The common chemical features include a high content of glycyl residues, amounting to nearly one-third of the total number of residues present in the purified preparations. This may be taken as indirect support for the recently proposed molecular models for the secondary structure of collagens (Ramachandran & Kartha, 1955; Cowan, McGavin & North, 1955; Rich & Crick, 1955), since these models predict that the crystalline regions of these proteins should contain at least this proportion of glycyl residues. Another common chemical feature is the presence of hydroxyprolyl

residues, which are found only in collagens, apart from a small amount in the protein elastin (Partridge & Davis, 1955). In each material listed in Table 4 there is a higher proportion of pyrrolidine residues than is commonly found in non-collagenous proteins. The collagens also have low contents of valine, leucine, isoleucine, phenylalanine, tyrosine, cystine and methionine.

The second part of Table 5 lists some contrasting features which distinguish the cuticular collagens of *Ascaris* and *Lumbricus* from other collagens. This classification is similar to that of Bear (1952), except that on the basis of the evidence presented in this publication it is seen that the collagen fibres from the Cuvierian tubules of *Holothuria forskali* now fall into the same group as the vertebrate fibres. The grounds for this classification now appear to have been widened from the single criterion used by Bear. It is not claimed that this scheme has any more general application than to the collagens specifically mentioned, nor that any of the features listed in it are necessarily connected, except for the relationship between the results of electron microscopy and low-angle X-ray-diffraction (Burge & Randall, 1955). On the one hand, it probably represents the closer relationship which is believed to exist between the chordates and the echinoderms than between the chordates and any other members of the Bilateria (Hyman, 1940). On the other hand, there may be chemical differences, distinguishable at the level of amino acid composition, between the collagen fibrils that show 640 Å periodicities and those that do not. It is interesting to note that the constancy in the total numbers of hydroxylic residues, found by Eastoe & Leach (1958) for a range of banded vertebrate collagens,

Table 5. *Summary of the properties of collagens*

All collagens for which complete amino acid analyses are now available have the following characteristics which may be used to distinguish them from other proteins:

1. A characteristic wide-angle X-ray-diffraction pattern (the definition of collagen).
2. An infrared-absorption band at 3330 cm.⁻¹.
3. A content of glycyl residues near to one-third of the total number of residues present.
4. Hydroxyprolyl residues.
5. High contents of pyrrolidine residues compared with other proteins.
6. Low contents of cystine, methionine, valine, leucine, isoleucine, phenylalanine, tyrosine and histidine.

	Vertebrate collagens and the fibres from the Cuvierian tubules of <i>H. forskali</i>	Cuticles of <i>Ascaris</i> and of <i>Lumbricus</i>
X-ray diffraction	Meridional low-angle pattern	No low-angle pattern yet observed
Electron microscopy	Fibrils banded in electron micrographs	Fibrils apparently not banded
Taxonomy	Deuterostomia	Protostomia
Chemical features	Hydroxylysyl residues About one hydroxylic side chain in every six residues Prolyl and hydroxyprolyl contents of the same order	No hydroxylysyl residues <i>Ascaris</i> cuticle has one hydroxylic residue in every 16 residues, and <i>Lumbricus</i> cuticle one in every three residues Prolyl and hydroxyprolyl contents of different orders

does not hold for the collagens without these periodicities.

In conclusion we may note that the shrinkage temperatures of the collagen fibres studied here are much too high to fall into line with the correlation in a wide range of vertebrate collagens between this property and the hydroxyproline content (Takahashi & Tanaka, 1953; Gustavson, 1957). This emphasizes the importance of other factors in the shrinkage process.

SUMMARY

1. The cuticle of *Ascaris lumbricoides* and the Cuvierian tubules of *Holothuria forskali* have been submitted to chemical analysis and some ancillary physical studies.

2. These tissues contain collagen, as evidenced by their wide-angle X-ray-diffraction patterns. Although their amino acid compositions are very different, they both have features which clearly relate them to the vertebrate collagens. A similar conclusion was reached in a study of earthworm cuticle (Watson, 1958a).

3. These collagens all contain hydroxyprolyl residues, and have a high content of glycyl residues and an infrared absorption band at 3330 cm.⁻¹.

4. Collagen-rich fractions obtained from these tissues have been analysed.

5. Contrary to previous belief, collagen fibrils from the Cuvierian tubules of *Holothuria forskali* have 640 Å periodicities along the fibril axis.

6. The cuticular collagens of *Lumbricus* and *Ascaris* do not have these periodicities, and in many respects their amino acid compositions are the most extreme yet encountered among the collagen proteins. One qualitative difference is that the cuticular collagens appear to lack hydroxylsyl residues.

7. The shrinkage temperatures of the collagens studied here cannot be directly related to their hydroxyproline contents.

We wish to thank Professor J. T. Randall, F.R.S., for directing this research and Dr H. B. Fell, F.R.S., for much advice. Thanks are due to Mr G. R. Wilkinson for the infrared spectra, to Mr G. F. Elliott for the low-angle X-ray-diffraction photograph and to Dr R. E. Burge for valuable discussions. We also wish to thank A. G. Ward, J. E. Eastoe and A. A. Leach of the British Gelatine and Glue Research Association for a helpful discussion. One of us (N.R.S.) acknowledges the receipt of a maintenance grant from the Department of Scientific and Industrial Research.

REFERENCES

- Astbury, W. T. (1938). *Trans. Faraday Soc.* **34**, 378.
 Astbury, W. T. (1939). *Annu. Rev. Biochem.* **8**, 113.
 Bailey, K. (1937). *Biochem. J.* **31**, 1141.
 Bear, R. S. (1952). *Advanc. Protein Chem.* **7**, 69.
 Bird, A. F. (1956). *Exp. Parasit.* **5**, 350.

- Bird, A. F. (1957). *Exp. Parasit.* **6**, 383.
 Bird, A. F. & Deutsch, K. (1957). *Parasitology*, **47**, 319.
 Burge, R. E. & Randall, J. T. (1955). *Proc. Roy. Soc. A*, **233**, 1.
 Chibnall, A. C., Rees, M. W. & Williams, E. F. (1943). *Biochem. J.* **37**, 354.
 Chitwood, B. G. (1936). *Proc. helm. Soc. Wash.* **3**, 39.
 Chitwood, B. G. & Chitwood, M. B. (1937). *An Introduction to Nematology*, part 1, pp. 30–32. Baltimore: Monumental Printing Co. Ltd.
 Cowan, P. M., McGavin, S. & North, A. C. T. (1955). *Nature, Lond.*, **176**, 1062.
 Dustin, J. P., Czajkowska, C., Moore, S. & Bigwood, E. J. (1953). *Analyt. chim. acta*, **9**, 256.
 Eastoe, J. E. (1954). *Nature, Lond.*, **173**, 540.
 Eastoe, J. E. & Leach, A. A. (1958). In *Recent Advances in Gelatin and Glue Research*, p. 173. Ed. by Stainsby, G. London: Pergamon Press.
 Fauré-Fremiet, E. & Garraut, H. (1944). *Bull. biol.* **78**, 206.
 Franks, A. (1954). Ph.D. Thesis: University of London.
 Gustavson, K. H. (1957). In *Connective Tissue*, p. 185. Ed. by Tunbridge, R. E. Oxford: Blackwell.
 Hyman, L. H. (1940). *The Invertebrates*, vol. 1, p. 131. New York: McGraw Hill.
 Marks, M. H., Bear, R. S. & Blake, C. H. (1949). *J. exp. Zool.* **111**, 55.
 Moore, S. & Stein, W. H. (1950). *Cold Spr. Harb. Symp. quant. Biol.* **14**, 179.
 Moore, S. & Stein, W. H. (1951). *J. biol. Chem.* **192**, 663.
 Moss, J. (1955). *Biochem. J.* **61**, 151.
 Partridge, S. M. & Davis, H. F. (1955). *Biochem. J.* **61**, 21.
 Picken, L. E. R., Pryor, M. G. M. & Swann, M. N. (1947). *Nature, Lond.*, **159**, 434.
 Ramachandran, G. N. & Kartha, G. (1955). *Nature, Lond.*, **176**, 915.
 Rich, A. & Crick, F. H. C. (1955). *Nature, Lond.*, **176**, 915.
 Rudall, K. M. (1955). *Symp. Soc. exp. Biol.* **9**, 49.
 Takahashi, T. & Tanaka, T. (1953). *Bull. Jap. Soc. sci. Fish.*, **19**, 603.
 Tomlin, S. G. & Worthington, C. R. (1956). *Proc. Roy. Soc. A*, **235**, 189.
 Walker, P. M. B. (1955). *Exp. Cell Res.* **8**, 567.
 Watson, M. R. (1958a). *Biochem. J.* **68**, 416.
 Watson, M. R. (1958b). In *Recent Advances in Gelatin and Glue Research*, p. 179. Ed. by Stainsby, G. London: Pergamon Press.
 Windrum, G. M., Kent, P. W. & Eastoe, J. E. (1955). *Brit. J. exp. Path.* **36**, 49.

EXPLANATION OF PLATE 5

X-ray-diffraction photographs and electron micrograph of the Cuvierian tubules of *Holothuria forskali*

- (a) Wide-angle X-ray-diffraction photograph. Fibre axis is vertical. Philips microcamera, with specimen–film distance of 1.5 cm., and a 40 μ collimator were used.
 (b) Low-angle X-ray-diffraction photograph of material stained with phosphotungstic acid. Fibre axis is vertical. The camera was based on the design of Franks (1954).
 (c) Electron micrograph of a collagen fibril teased from a tubule, stained in 0.1% phosphotungstic acid in water and examined in a Metropolitan-Vickers EM 3A microscope.

(a)

(b)

(c)

M. R. WATSON AND N. R. SILVESTER—STUDIES OF INVERTEBRATE COLLAGEN PREPARATIONS

(Facing p. 584)