ing evidence is produced. We wish to emphasize that all the species mentioned above were described long after Simpson's publication and that we only feel that it is necessary to record this correction because of the frequent uncritical references of other authors to the Brora Coal. It may still be proved that there were pre-Cretaceous angiosperms, but this particular evidence must be discarded.

N. F. HUGHES

Sedgwick Museum,
Cambridge.

R. A. COUPER

New Zealand Geological Survey.
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Occurrence of Collagen in the Phylum Mollusca

CONSIDERABLE interest has recently been shown in the occurrence of a collagen type of protein among the various invertebrate groups. X-ray diffraction[1,2] studies have explored a wide range of examples, and more recently chemical investigations have used various chromatographic techniques to determine the composition of certain preparations[3–5]. Most of this work has been performed on tissues from single species bearing little or no relationship to one another. In the present study, the occurrence of collagen has been investigated in representatives of the three major classes of the phylum Mollusca, using the occurrence of hydroxyproline as an indication of the presence of a collagen-type protein. The examples were: class Gastropoda, *Helix aspersa* (garden snail); class Lamellibranchiata, *Mytilus edulis* (edible mussel); class Cephalopoda, *Loligo vulgaris* (common squid).

The dead animals were washed in isotonic saline for several days, rinsed with distilled water and blotted dry with filter paper Moisture content, which was close to 85 per cent for all the samples, was estimated by drying to constant weight at 105° C. and the ash was measured by igniting at 550°. Hydroxyproline was determined by a modification[6] of the Neuman and Logan[7] procedure and total nitrogen by Kjeldahl determination. Results have been expressed in terms of the ratio of hydroxyproline nitrogen to total nitrogen. Analyses were performed on the whole snail and mussel and on the parts of the animals indicated in Table 1. Determinations on the squid were carried out on various regions and not on the whole animal. The byssus apparatus and ctenidia were dried *in vacuo* over phosphorous pentoxide before analysis was carried out. The results in Table 1 show the presence of hydroxyproline and hence of collagen in almost all samples examined, with a tendency to be concentrated into specific structures. This is particularly shown for the snail, where the collagen is concentrated in the body-wall.

About 240 gm. of mussel bodies were extracted with hot water at *p*H 4·5 using higher temperatures for each successive extract (60–90° C.). The material containing hydroxyproline went preferentially into solution, the mean hydroxyproline nitrogen to total nitrogen ratio (as per cent) being 0·76 for the extracts.

Table 1. OCCURRENCE OF HYDROXYPROLINE IN THREE SPECIES OF MOLLUSCA

Species	Sample	Ash (percentage in dry material)	Percentage in dry, ash-free material		Hydroxyproline nitrogen/total nitrogen (per cent)
			Hydroxyproline	Total nitrogen	
Helix aspersa	Whole animal	13	0·94	7·75	1·30
	Body-wall		1·91	9·5	2·16
	Viscera		Trace		
Mytilus edulis	Whole animal	8	0·48	10·3	0·49
	Byssus apparatus		1·50	8·4	1·9
	Ctenidia		0·60	8·0	0·80
Loligo vulgaris	Mantel wall	23	0·43	16·1	0·29
	Fin	11	1·06	11·5	1·00
	Cranial cartilage	12	4·15	9·3	4·80
	Head–foot*	15	0·82	12·5	0·70

* One arm together with associated part of head

Approximately 50 gm. of snail bodies (dry weight about 8 gm.) were soaked for a short time in 5 per cent hydrochloric acid, followed by numerous changes of ½ per cent sodium chloride both before and after removal of the viscera, for a week. Preliminary experiments had shown that a short treatment of the snail body-wall with saturated lime water increased the rate of extraction of hydroxyproline-rich material in warm water. Four changes of lime water were given during four days. The body-wall material was washed and extracted at 70° C., 2 hr., 80° C., 2 hr. and 90° C., 2 hr. The first extraction was lost in an unsuccessful attempt to fractionate it. The second and third extracts were filtered and concentrated and dried over phosphorus pentoxide. The second extract was observed to set to a gel at 4° C. after concentration. The analytical results for these extracts are given in Table 2.

Table 2. ANALYSIS OF EXTRACTS FROM LIMED SNAIL BODY-WALLS

Extract	Yield (mgm.)	Ash (per cent)	Percentage in dry, ash-free material		Hydroxyproline nitrogen/total nitrogen (per cent)
			Hydroxyproline	Total nitrogen	
2	112	4·0	10·8	16·6	6·95
3	200	3·6	12·6	17·8	7·6

It was noted that the hydrolysate of both extracts was light in colour and resembled that for gelatin samples known to be largely free of polysaccharide. The hydroxyproline and total nitrogen figures are therefore likely to be close to those of the intact collagen of the snail body-wall.

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S. C. MELNICK

The British Gelatine and
Glue Research Association,
2a Dalmeny Avenue,
Holloway, London, N.7.
March 27.

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