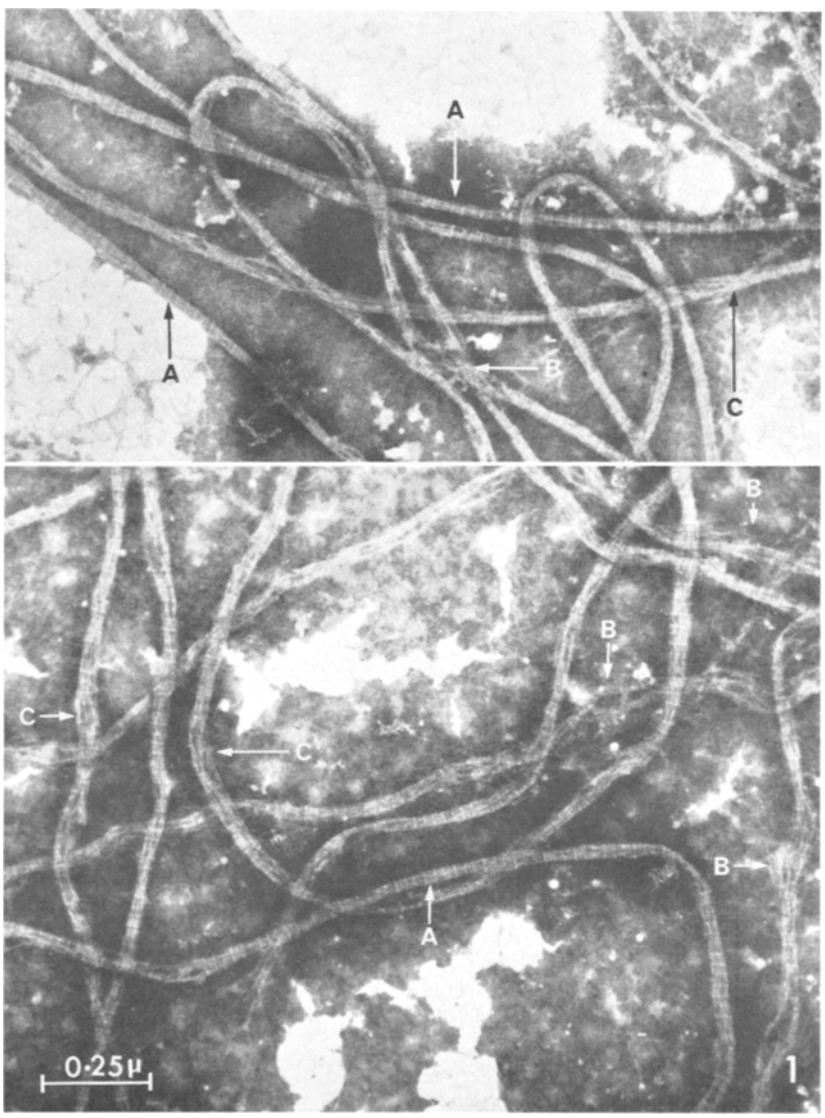
Polymeric Collagen Isolated from Squid *(Loligo peallii)* Connective Tissue

STEVEN and his co-workers have adopted and developed with considerable success the Nishihara technique for the solubilization from tissues of collagen in polymeric form[1-6]. The method which involves pretreatment of the tissue with crude bacterial α-amylase has been applied to various tissues including skin, tendon, intervertebral disc, cornea and intestinal submucosa but has not yet been applied to invertebrate connective tissues. The present report is a preliminary account of the extraction of a polymeric collagen from the mantle wall tissue of the squid *Loligo peallii* using the Nishihara method.

Materials and methods. Squid mantle wall tissue was homogenized in 5% w/v sodium chloride solution at 4 °C. The homogenate was centrifuged and the insoluble component washed in 0.5 *M* NaCl by repeated centrifugation until the supernatant was clear and protein-free. The residue was washed exhaustively with distilled water and then digested with bacterial α-amylase[2,7] in 0.22 *M* phosphate buffer at pH 5.4 for 4 days at 4 °C. During digestion the homogenate was transformed from an even suspension to flocullated masses.

The digest was centrifuged down, washed repeatedly with 5% sodium chloride followed by water and then homogenized in a large volume of 0.2 *M* acetic acid. The suspension was stirred for 24 h and then centrifuged at 300 *g* for 20 min. The opalescent viscous supernatant contained virtually all of the enzyme-digested homogenate a small residue remaining. Only a small percentage (less than 10%) of the total collagen of the squid body wall is extractable by neutral salt solution or acid buffer without α-amylase pretreatment[8]. Samples were taken for electron microscopy from this dispersate. Addition of sodium chloride to the dispersate to a concentration of 5%, or adjustment of the pH to 7.0, produced a mass of collagen fibres which could be wound onto a glass rod. Samples for amino acid and carbohydrate analysis were repeatedly precipitated in this manner from 0.2 *M* acetic acid before washing and drying from acetone. Amino acid analyses were carried out on a Technicon analyser. Analyses of carbohydrates were carried out by a combination of colorimetric methods and gas-liquid chromatography[9].

Results and discussion. Electron microscopy of the dispersed collagen fibres negatively stained with phosphotungstic acid (Figure) showed fibrils with the 680 Å repeat striation typical of collagen. The fibrils are un-



Squid mantle wall polymeric collagen fibrils negatively stained with phosphotungstic acid (pH 7.0) × 80,000, showing thin fibrils with 680 Å periodicity (A), swollen stocking-like regions (b) and regions which while swollen still show characteristic striations (C).

usually narrow (300 Å diameter) and the striations are rather weak although the fibrils appear clean. Narrow fibrils of polymeric collagen resembling these have been noted elsewhere[6,10] and in the present case there is evidence of the partial unravelling of fibrils to show the 'stocking' type structure of protofibrils noted by STEVEN in intestinal submucosal polymeric collagen[6].

Amino acid compositions of invertebrate collagens are more varied than those of vertebrate materials. That of the squid collagen isolated here is broadly typical (Table) of invertebrate collagens although the glycine content is lower than might be expected of contaminant-free collagen. The hydroxyproline, proline and hydroxylysine values are within the ranges found in other invertebrate collagens[11]. No 3-hydroxyproline was detected. The hydroxyproline content estimated colorimetrically is rather low (5.35% of the weight) which is suprising in view of the clean electron microscopic appearance of the material and the low hexosamine content (below).

The squid collagen contains 4.42% neutral sugar and 0.032% hexosamine. The neutral sugar is mainly glucose and galactose in 1:1 molar ratio with small amounts of mannose and fucose and a trace of xylose. The quantity of neutral carbohydrate present is greater than in most vertebrate collagens but is lower than in many invertebrate collagens[11]. The presence of glucose and galactose or galactose alone, as predominant monosaccharides, is a characteristic feature of vertebrate and invertebrate collagens[12,13].

The reasons for the indistinctness of the banding pattern in the collagen fibres is not clear; this may be connected with the moderately elevated carbohydrate content as may be the thinness of the fibrils[9]. It is of interest to note that thin polymeric collagen fibres closely resembling the present ones can be isolated from vertebrate cornea[10]; both cornea and squid skin contain the unusual unsulphated mucopolysaccharide chondroitin[14,15]. The lack of strong banding may however indicate conformational abnormality of the tropocollagen; the IR-spectrum shows that the N-H stretching mode has its peak at the unusually low value of 3290 cm^{-1} in contrast to 3330 cm^{-1} for most collagens. This observation would suggest that the stabilizing hydrogen bonds are shorter than normal[16].

The Nishihara method can thus be equally applied to preparation of insoluble collagen in improved yields from vertebrate and invertebrate tissues.

Amino acid composition of squid mantle wall polymeric collagen

Residue	Composition	Residue	Composition
Hydroxyproline	90	Isoleucine	17
Aspartic acid	68	Leucine	38
Threonine	33	Tyrosine	10
Serine	72	Phenylalanine	18
Glutamic acid	88	Hydroxylysine	16
Proline	78	Lysine	21
Glycine	298	Histidine	7
Alanine	75	Arginine	48
Valine	23		

Amino acids in residues per 1000 total residues. Serine, threonine and tyrosine corrected for hydrolytic losses.

Résumé. On a isolé du collagène naturel et polymère du tissu du manteau du calmar *Loligo peallii* par la méthode de Nishihara, les fibres présentant au microscope électronique une périodicité de 680 Å. Le collagène a une composition typique d'amino acides et contient l'hydrate de carbone dans une proportion restreinte; principalement sous forme de glucose et de galactose.

S. HUNT[17], M. E. GRANT and S. J. LIEBOVICH

Departments of Biological Chemistry, Medical Biochemistry and The Rheumatism Research Centre, University of Manchester, Manchester (England), 19 May 1970.

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[17] Present address: Department of Biological Sciences, University of Lancaster, Lancaster (England).

Structure of Bradykinin-Potentiating Peptide Containing Tryptophan from the Venom of *Agkistrodon halys blomhoffii*

In a previous paper[1], we have reported on the isolation of 5 bradykinin-potentiating peptides (potentiators A, B, C, D and E) and an examination of their amino acid composition. These peptides potentiated the bradykinin action on guinea-pig ileum in vitro. Out of these five peptides, the amino acid sequence of the potentiator B has been determined to be as follows[2]:

Pyr-Gly-Leu-Pro-Pro-Arg-Pro-Lys-Ile-Pro-Pro

This paper describes the amino acid sequence of the potentiator E, which contains tryptophan and has an amino acid composition which is dissimilar to that of the other potentiators A, B, C and D.

The N-terminal amino acid of the potentiator E was not detected by Edman degradation, but a C-terminal amino acid was found, by hydrazinolysis, to be proline. From the tryptic hydrolysate of potentiator E, 2 peptide