Fibrous Protein Ultrastructure of Gastropod Periostracum (*Buccinum undatum* L.)

We wish to report here some ultrastructural studies which have revealed an unusual fibrous protein, resembling fibrinogen, organized in a highly distinctive manner to form the periostracum of a gastropod mollusc.

The shells of most bivalve and gastropod molluscs are covered by an outer protective layer of quinone-tanned protein called the periostracum[1]. Examination of the superficial appearance, texture and mechanical properties of the thicker periostraca, such as are found in the larger mussels and whelks, might lead one to the intuitive conclusion that these materials are perhaps elaborated from fibrous protein sub-units. In spite of this, studies of periostraca to date, made at the ultrastructural level, have indicated little other than a relatively simple organization of somewhat amorphous constituents[2–4]. Such studies have however been confined to the periostraca of bivalve molluscs.

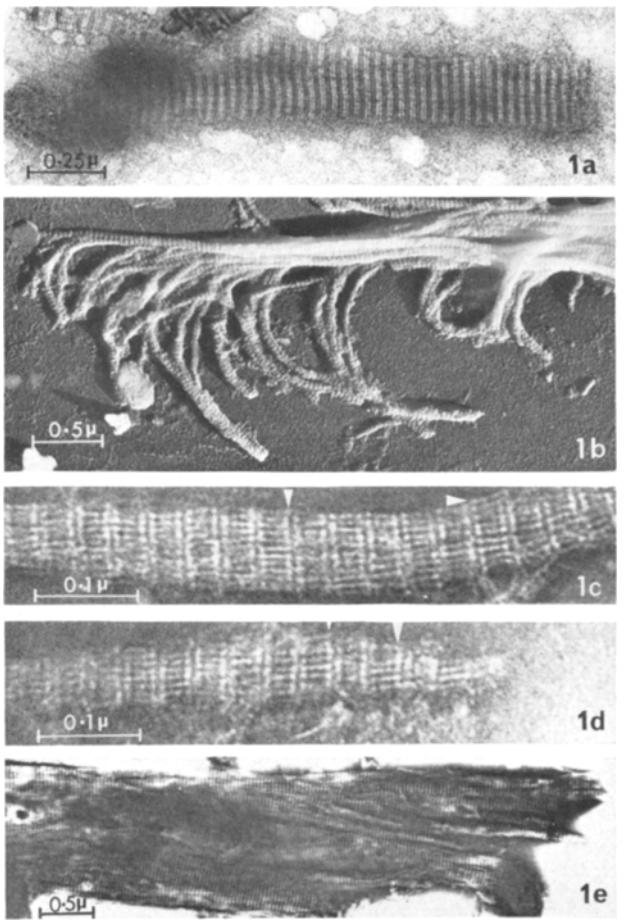

Fig. 1. a) Electron micrograph of sonicated *Buccinum undatum* periostracum negatively stained with phosphotungstic acid pH 5.6. × 63,000.
b) Electron micrograph of sonicated *B. undatum* periostracum shadowed with Au/Pd. × 28,000.
c) High resolution electron micrograph of *B. undatum* periostracal ribbon negatively stained with phosphotungstic acid pH 5.6 showing detail of the sub-unit structure. Arrows indicate points of shear and ends of sub-units. × 210,000.
d) As for Figure 1, c but showing the broken end of a ribbon. Arrows indicate ends of sub-units. × 210,000.
e) Electron micrograph of sheet of *B. undatum* periostracal ribbons in a larger fragment of sonicated periostracum. Negatively stained with phosphotungstic acid pH 5.6. × 21,000. Contrast in Figures a, c, d and e was improved by use of the 'out of focus masking' technique.

The periostracum of the Stenoglossan marine gastropod *Buccinum undatum* L. is a brownish, tough, horny material often as much as 1000 μ thick. Preliminary fragmentation of this substance by maceration followed by long periods (30 min) of disruption in an ultrasonic disintegrator yielded sufficiently finely divided aqueous suspensions, of the constituent structural proteins, for examination in the electron microscope. These structural proteins, when negatively stained or shadowed, can be seen to consist of well-defined ribbon-like units with a distinctive longitudinal banding pattern (Figures 1, a and b). Examination of the negatively stained preparation shown in Figure 1, a indicates a fibre axis repeat of the order of 350 Å. Within each repeat unit there is a very marked double band some 150 Å wide while between each set of these double bands there can be discerned a much fainter second pair of bands separated on either side from the larger bands by a distance of about 50 Å and separated from oneanother by a distance of about 34 Å. Each repeat unit has an axis of symmetry normal to the long axis of the fibre or ribbon. The basic fibrils, from which the ribbons appear to be assembled by lateral aggregation, can be clearly distinguished and are separated laterally by a centre to centre distance of about 70 Å. More detailed examination of the ribbons (Figure 1, c) suggests that the prominent features (unstained in the negatively stained preparations) are globular units probably united by rod-shaped regions. Our observations of the terminal regions of the ribbons, where breaks have occurred (Figure 1, d) or regions where the ribbons have sheared (Figure 1, c), leads us to postulate that the basic sub-unit of the ribbon is that shown in Figure 2, i.e. a dumbell-like molecule consisting of 2 large spherical or globular nodules at the extremities of a slender filament along which are distributed 2 smaller semispheres, symmetrically about the centre of the long axis of the unit. This model of the basic periostracal protein unit would resemble that which has been suggested for the fibrinogen molecule[5] while the striated periostracal protein ribbons might also be likened to the fibrin fibre in the end to end and side by side aggregation of the sub-units. Presumably the periostracal ribbons are stabilized by some form of cross-linking between the sub-units.

The ribbons themselves seem to be laterally aggregated in vivo into extensive sheet-like structures of limited thickness. This is indicated by the appearance of larger fragments when seen in the electron microscope (Figure 1, e) but also by the appearance of sections of the intact periostracum.

The extremely tough nature of the periostracum has led to excessive difficulty in sectioning fixed and embedded material. However the section shown in Figure 3, cut with a diamond knife in a plane perpendicular to the surface of the periostracum, indicates a complex lamellar

[1] E. T. DEGENS, D. W. SPENCER and R. H. PARKER, Comp. Biochem. Physiol. *20*, 553 (1967).
[2] S. KAWAGUTI and N. IKEMOTO, Biol. J. Okayama Univ. *8*, 21 (1962).
[3] I. K. KAWAKAMI and G. YASUZUMI, J. Electron Microsc. *13*, 119 (1964).
[4] G. BEVELANDER and H. NAKAHARA, Calc. Tiss. Res. *1*, 55 (1967).
[5] C. E. HALL and H. S. SLAYTER, J. Biophys. Biochem. Cytol. *5*, 11 (1959).

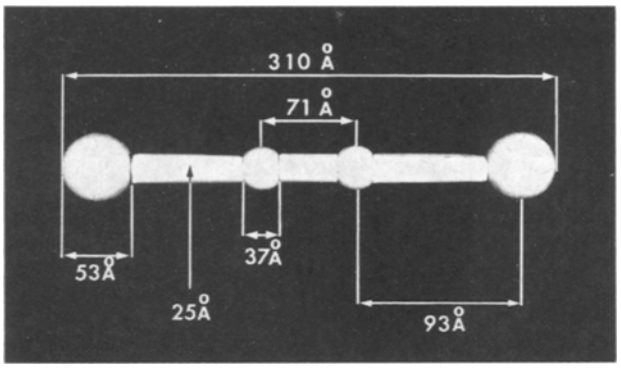

Fig. 2. Proposed model for the structural protein sub-unit of *B. undatum* periostracum.

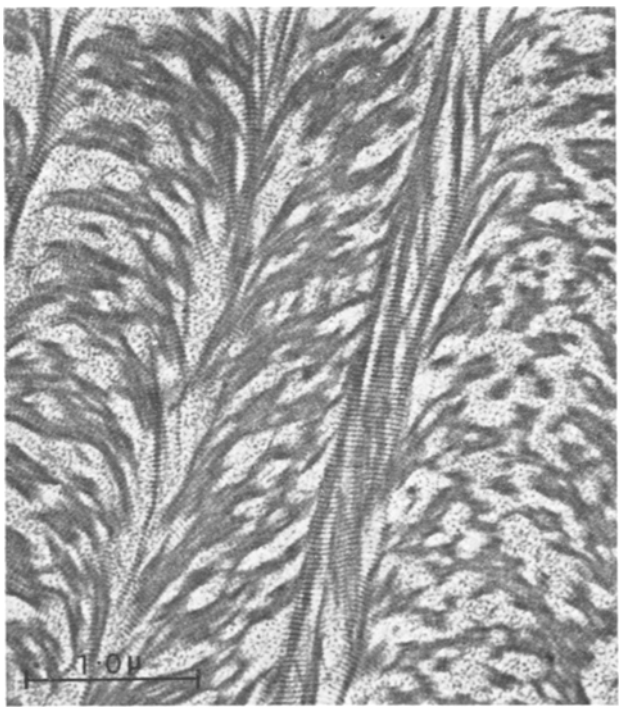

Fig. 3. Electron micrograph of a section of *B. undatum* periostracum made in a plane perpendicular to the surface of the periostracum (horizontal in this picture). Fixed in 5% glutaraldehyde pH 7.0 cacodylate buffer, 0.2 *M* sucrose. Post-fixed in osmic acid and embedded in Epon. Stained with uranyl acetate and Reynold's lead stain. × 35,000.

structure built up from ribbons in which features of the striated pattern already described can be clearly seen.

This type of parabolic lamellar structure, with the appearance of the flowering of systems of bundles or sheets of fibres, has been noted elsewhere; principally in arthropod cuticle[6,7] but also in the tunic of tunicates[8]. A similar phenomena has been noted for certain liquid crystal systems[9] and in the globules of oothecal structure protein secreted into the lumen of the left collateral gland of the praying mantis[10].

An explanation of this type of parabolic lamellar structure has been proposed[6] on the basis of Moiré patterns formed from fibril systems seen in oblique section. These systems consist of superimposed sheets of fibres, each sheet having all of the individual fibre axes parallel, and with every sheet rotated through a small angle relative to that above it. A regular progression of rotation, always in the same sense, down the stack of sheets is proposed. Oblique sectioning through such a stack gives rise to the artefact of parabolic lamellae as has been demonstrated by model building[10].

On the basis of the available evidence therefore we suggest that the periostracum of this gastropod is organized from stacked sheets of ribbons or fibres, composed from small asymmetric sub-units as described above, in which the mean fibre axis of each sheet is specifically rotated relative to that of the sheets immediately above and below it and in which the plane of the sheets is parallel to the surface of the periostracum[11].

Résumé. Le périostracum du gastéropode *Buccinum undatum* L. se compose de rubans de protéine fibreuse disposés en lames formant des stries répétées. Ces rubans sont accolés et joints bout à bout. Leurs éléments constitutifs (subunités) sont en forme de haltères ressemblant à la molécule de fibrinogène.

S. Hunt and K. Oates

Department of Biological Sciences, University of Lancaster, Bailrigg, Lancaster (Gt. Britain), 8 May 1970.

[6] Y. Bouligand, C. r. hebd. Séanc. Acad. Sci. Paris *261*, 3665 (1965).
[7] M. Locke, J. Biophys. Biochem. Cytol. *10*, 589 (1961).
[8] A. C. Neville, Adv. Insect Physiol. *4*, 213 (1967).
[9] C. Robinson, Molec. Crystals *1*, 467 (1966).
[10] W. Kenchington and N. E. Flower, J. Microsc. *89*, 263 (1969).
[11] We thank the Department of Physics at the University of Lancaster for providing facilities of their electron microscope.

In vivo Rupture of the Imidazole Ring of Histamine

Histamine is catabolized through various pathways in mammalian tissues but the imidazole ring always remains intact. When the substance is injected into animals it is largely excreted in the urine in the form of various metabolites[1]. Schayer[2] injected two rats and a guinea-pig with about 0.1 μg of ring-$^{14}C_2$-histamine per g and detected no radioactivity in the expired air. In a forth animal, injected with about 10 times this quantity of $^{14}C_2$-histamine he detected minute quantities of $^{14}CO_2$ which he thought might be due to an impurity in the histamine used.

We have recently done some experiments which suggest that in ruminant animals the imidazole ring of dietary histamine is ruptured. In such animals the food is thoroughly fermented by micro-organisms in the rumen before it passes to the intestines. Previous work has shown that when $^{14}C_2$-histamine and carrier histamine were given by mouth the biological activity disappeared