The fin musculature of cuttlefish and squid (Mollusca, Cephalopoda): morphology and mechanics

WILLIAM M. KIER

Department of Biology, CB 3280 Coker Hall, University of North Carolina, Chapel Hill, North Carolina 27599-3280, USA

(With 5 plates and 1 figure in the text)

The lateral fins of cuttlefish and squid consist of a tightly packed three-dimensional array of musculature that lacks bony skeletal support or fluid-filled cavities for hydrostatic skeletal support. During swimming and manoeuvring, the fins are bent upward and downward in undulatory waves. The fin musculature is arranged in three mutually perpendicular planes. Transverse muscle bundles extend parallel to the fin surface from the base of the fin to the fin margin. Dorso-ventral muscle bundles extend from dorsal and ventral connective tissue fasciae to a median connective tissue fascia. A layer of longitudinal muscle bundles is situated adjacent to both the dorsal and ventral surface of the median fascia. The muscle fibres are obliquely striated and include a core of mitochondria. A zone of muscle fibres with a more extensive core of mitochondria is present in both the dorsal and the ventral transverse muscle bundles. It is hypothesized that these muscle masses include two fibre types with different aerobic capacity. A network of connective tissue fibres is present in the transverse and dorso-ventral muscle masses. These fibres, probably collagen, are oriented at 45° to the long axes of the transverse and dorsoventral muscle fibres in transverse planes.

A biomechanical analysis of the morphology suggests that support for fin movements is provided by simultaneous contractile activity of muscles of specific orientations in a manner similar to that proposed for other 'muscular-hydrostats'. The musculature therefore provides both the force and support for movement. Connective tissue fibres may aid in providing support and may also serve for elastic energy storage.

Contents

Introduction

The musculoskeletal system of many molluscs, especially cephalopods, is characterized by a tightly packed, three-dimensional array of musculature (Kier, 1988). Previous research on the biomechanics of this complex muscular array in cephalopods has focused on the arms and tentacles of squid (Kier, 1982, 1985), the cirri of nautilus (Kier, 1987), the arms of octopus (Tittel, 1961, 1964; Kier, 1988) and the mantle of squid and cuttlefish (Ward, 1972; Ward & Wainwright, 1972; Packard & Trueman, 1974; Bone, Pulsford & Chubb, 1981; Mommsen et al., 1981; Gosline & Shadwick, 1983a, b; Gosline, Steeves, Harman & Demont, 1983). This paper examines the functional morphology of the musculature of the lateral fins of cuttlefish and squid. The fins are of particular interest because they also consist of a tightly-packed, three-dimensional array of musculature, yet their movements are different from those of the arms, tentacles and mantle; the fins produce rhythmic undulatory waves used in locomotion and hovering. This stereotyped movement may make them particularly amenable to experimental analysis.

In this paper, the morphology and ultrastructure of the musculature of the lateral fins of the cuttlefish, *Sepia officinalis* is examined and compared to that of two squid species, *Loligo forbesi* and *Sepioteuthis sepioidea*. The morphology is then analysed from the standpoint of biomechanics in order to predict the functional role of the various muscle arrangements in producing movement and providing skeletal support. These predictions will be tested experimentally in a separate study in preparation.

Materials and methods

Specimens of *Sepia officinalis* and *Loligo forbesi* were supplied by the Laboratory of the Marine Biological Association of the United Kingdom, Plymouth. *Sepia officinalis* specimens were also supplied by the Stazione Zoologica, Naples, Italy. Specimens of *Sepioteuthis sepioidea* were captured at Carrie Bow Cay, Belize as outlined by Kier (1982). Blocks of fin tissue were obtained from freshly killed specimens that were initially anaesthetized with magnesium chloride (Messenger, Nixon & Ryan, 1985) or 1% ethanol in sea water. Tissue examined by light microscopy was fixed in Bouin-Duboscq fixative or in 10% formalin in sea water for 24-48 h, dehydrated in ethanol and embedded in polyester wax (MP 37 °C), (Steedman, 1957) or paraffin (MP 56 °C). The tissue blocks were serially sectioned at 5-10 μm in 3 mutually perpendicular planes and stained with either Milligan Trichrome Stain (Milligan, 1946), Picro-Ponceau with Weigert Iron Hematoxylin (Gurr, 1956) or Verhoeff Elastin Stain (Mallory, 1944) using procedures outlined by Humason (1979). *Sepia* fin sections were also stained with Palmgren's silver stain toned with gold chloride (Palmgren, 1948, 1955, 1960) using procedures outlined by Bone (1972). Stained and unstained sections were examined with brightfield, phase contrast and polarized light microscopy. In addition, portions of the formalin-fixed *Sepia* and *Sepioteuthis* fin material were embedded in glycol methacrylate plastic (Historesin, LKB Produkter, AB), sectioned at 0·5-2·5 μm and stained with Lee's methylene blue-basic fuchsin stain (Bennett et al., 1976).

Tissue examined by transmission electron microscopy was fixed in 3·0% glutaraldehyde, 0·065% phosphate buffer, 0·5% tannic acid and 6% sucrose for 6-8 h, rinsed in buffer overnight and post-fixed in a 1:1 mixture of 2% osmium tetroxide and 2% potassium ferrocyanide in 0·13M cacodylate buffer. The tissue was dehydrated in ethanol, embedded in epoxy resin (Taab 812 resin, Taab Laboratories, Reading, UK). Sections of silver-grey interference colour were stained with saturated aqueous uranyl acetate and with Reynolds' (1963) lead citrate and were examined with a Zeiss EM 10CA electron microscope.
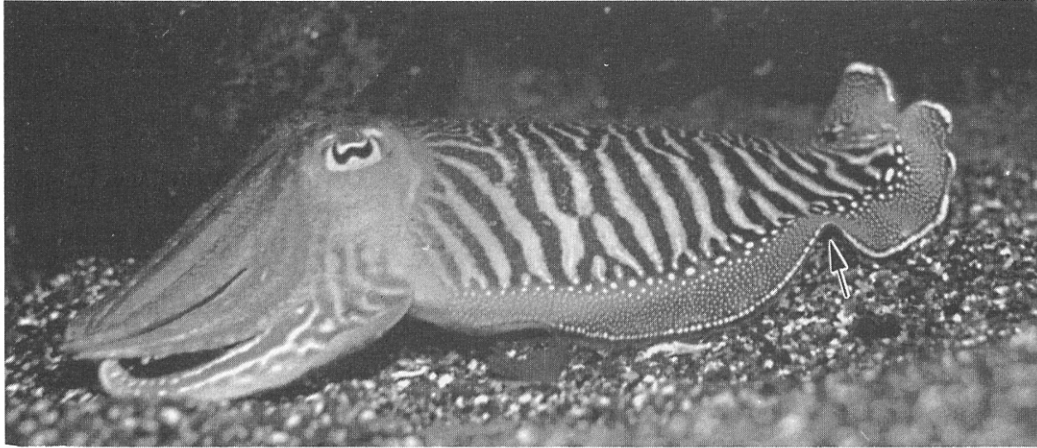
PLATE I. Photograph showing single undulatory wave (arrow) on left fin of *Sepia officinalis* resting on bottom of aquarium.

The relative areas of the mitochondria and myofilaments of the muscle cells of the fin were measured on electron micrographs of transverse sections of cells. A series of 5–7 micrographs of adjacent portions of the section were assembled into a composite and the composite was placed on a digitizing tablet (GTCO Digi-Pad 5) interfaced with a microcomputer. The outlines of the mitochondrial core and cell were traced for each complete cell cross-section using *Sigma-Scan* software (Jandel Scientific, Corte Madera, CA). More than 25 cell profiles were included in each composite. Statistical comparisons were made between various cell parameters using routines included in *Sigma-Scan*.

Fin movements were photographed from the side, front and top in aquaria.

Results

Gross morphology of the fins

The general form of the fins of *Sepia officinalis* is shown in Plate I. The fins extend laterally from each side of the mantle along its entire length. They are thickest at their base where they attach to the mantle and taper toward their edges. The edge of a fin is parallel to its base and the length is approximately ten times the width. When the cuttlefish swims or hovers, undulatory waves travel along the length of the fin. Although much of the thrust for locomotion is produced by jets of water from the mantle cavity, the fins probably aid in producing thrust at low swimming speeds and in providing stability (Russell & Steven, 1930; Bidder & Boycott, 1956; Boycott, 1958). As a wave passes, adjacent segments of the fin bend serially upward and then downward. Waves are initiated at either the anterior or posterior end of the fin. Waves passing from anterior to posterior provide thrust for forward movement, while those passing from posterior to anterior provide thrust for backward movement. When the animal is turning around a central vertical axis (yawing), undulatory waves passing from anterior to posterior on one side are often accompanied by waves of the opposite direction on the other side. In addition, hovering animals are sometimes observed to initiate undulatory waves at both the anterior and posterior ends of the fins simultaneously; the

waves travel toward one another and terminate where they meet in the middle of the fin. The amplitude and frequency of the waves is variable. When resting on the bottom or hovering, small amplitude waves are common. During feeding or rapid locomotion, a higher amplitude and frequency of undulatory waves is observed.

The general form of the fins of the loliginid squid species *Sepioteuthis sepioidea* is convergent upon those of the cuttlefish, *Sepia officinalis* in that they extend along the entire length of the mantle and the fin margin parallels the fin base. *Loligo forbesi*, also a loliginid, differs from *Sepia officinalis* and *Sepioteuthis sepioidea* because its fins do not extend along the entire length of the mantle and they are triangular in shape when viewed from above. This latter condition is more typical of loliginid squids. Systematic observations of fin movements of the two squid species were not made in this study.

Microanatomy of the fins

The fins of the cuttlefish, *Sepia officinalis* were the focus of this study. The microanatomy of the fins of the two loliginid squid species mentioned above was found to be virtually identical to that of *Sepia officinalis*. For the purpose of this discussion the sectional planes are defined as follows (Fig. 1): Transverse sectional planes are defined as vertical sections (with animal in the typical orientation assumed in life) perpendicular to the margin of the fin. Parasagittal sectional planes are vertical sections through the fin parallel to the long axis of the fin. Frontal sectional planes are horizontal sections in the plane of the fin.

Figure 1 is a schematic cutaway view of a portion of a fin of *Sepia officinalis* showing the muscles and associated structures. The bulk of the fin consists of a tightly packed three-dimensional array of musculature. The muscle fibres of this array principally have their origin and insertion on three connective tissue fasciae; the dorsal and ventral fasciae that lie immediately beneath the dermis on the dorsal and ventral surface of the fin, and a median fascia that divides the fin into dorsal and ventral portions (Fig. 1 & Plate II). The fasciae consist of layers of birefringent connective tissue fibres that exhibit staining reactions typical of collagen. The connective tissue fibres of the dorsal and ventral fascia, when viewed in grazing frontal sections, show a slight degree of preferred orientation in the longitudinal and transverse directions. The median fascia is a less compact layer of connective tissue fibres arranged as a feltwork and embedded in an amorphous matrix. Some of the fibres in the median fascia show staining reactions typical of elastin. If these fibres are indeed a rubber-like protein, they would not be expected to be birefringent. It was not possible, however, to determine whether or not they are birefringent because they are surrounded by birefringent collagen fibres.

The muscle fibres of the fin are arranged in three mutually perpendicular orientations. The dorsal and ventral transverse muscle bundles have their origin on a cartilaginous plate called the fin cartilage (Tompsett, 1939) located at the base of the fin along its entire length (Fig. 1). The transverse muscle bundles extend laterally from their origin toward the fin margin. As the bundles extend laterally, small groups of muscle fibres branch from the bundle to insert on the median connective tissue fascia (Plate IIb), thereby reducing the thickness of the bundles toward the fin margin. The transverse bundles also branch and connect with adjacent transverse bundles (Plate IIIa). The transverse muscle bundles are separated from one another by sheets of dorso-ventral muscle fibres. The dorso-ventral muscle fibres have their origin and insertion on the connective tissue fasciae of the fin. Muscle fibres of the dorso-ventral muscle sheets in the dorsal portion of the fin extend from the dorsal fascia to the median fascia, while the dorso-ventral muscles of the
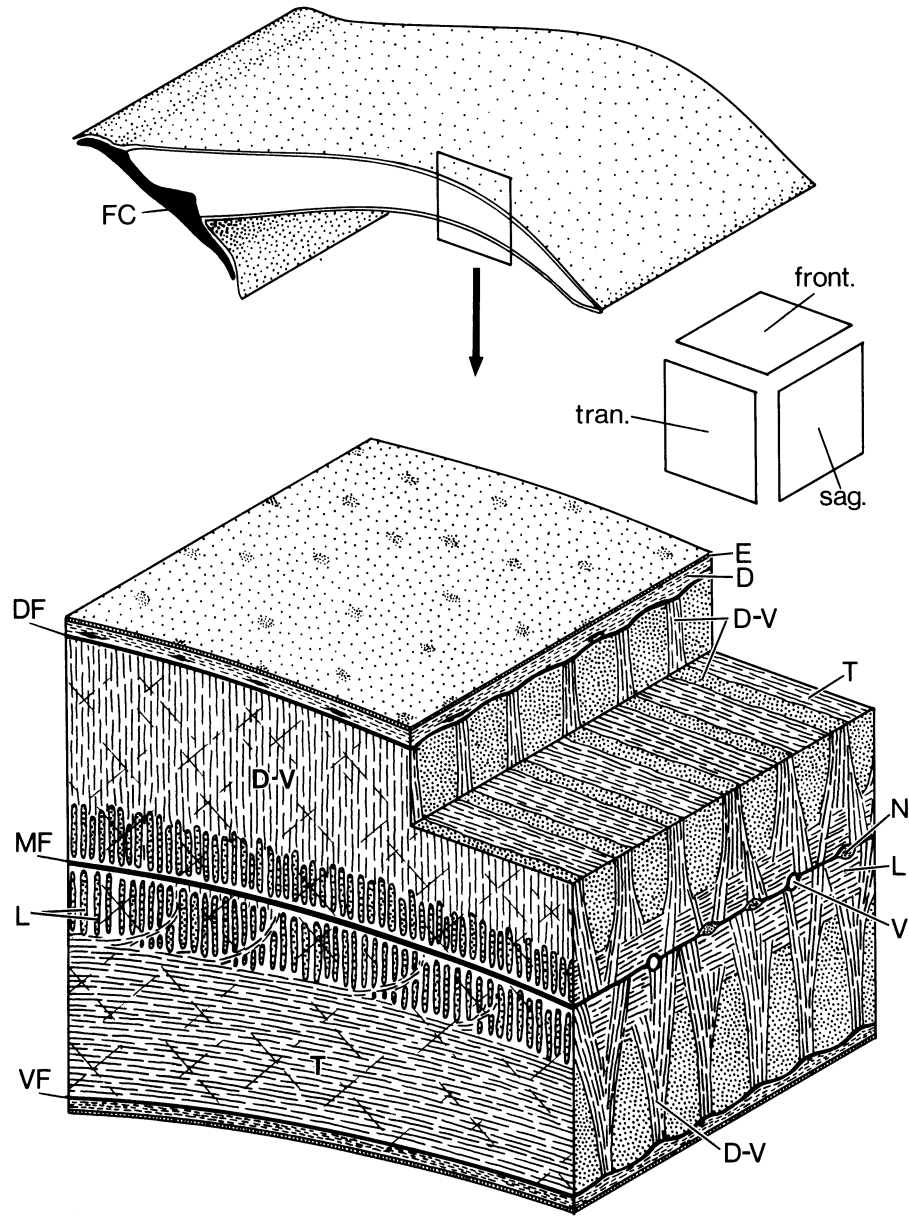
FIG. 1. Schematic diagram of the microanatomy of the fin of *Sepia officinalis*. The sectional planes (front., frontal; tran., transverse; sag., sagittal) are indicated. D, dermis; DF, dorsal fascia; D-V, dorso-ventral muscle; E, epidermis; FC, fin cartilage; L, longitudinal muscle; MF, median fascia; N, fin nerve; T, transverse muscle: V, blood vessel; VF, ventral fascia.

ventral portion of the fin extend from the ventral fascia to the median fascia. Dorso-ventral muscle fibres were not observed to extend across the median fascia, but connective tissue fibres were sometimes observed to cross the median fascia from its dorsal to ventral surface.

In addition to the transverse and dorso-ventral muscle fibres, a layer of longitudinal muscle fibres is situated adjacent to the dorsal and ventral surface of the median fascia. The ventral longitudinal muscle layer is thicker than the dorsal layer in the cuttlefish and squid species studied. The dorso-ventral muscle fibres extend through and divide the longitudinal muscle layers into bundles. The dorso-ventral muscles are thus arranged as transverse sheets of fibres between the transverse muscle bundles, but are divided into laterally compressed bundles as they extend through the longitudinal muscle layer to insert on the median fascia (Plates IIb and IIIa). The longitudinal muscle bundles often branch laterally to connect with adjacent longitudinal muscle bundles. The longitudinal muscle layers are less thick both along the base of the fin adjacent to the cartilage and along the fin margin. Grazing frontal sections show that the dorsal and ventral longitudinal muscle layers are not always precisely parallel (Plate IIIa). In some sections, their long axes were oriented at an angle of approximately 10-15° to one another.

In addition to the three connective tissue fasciae, connective tissue fibres were also observed within the muscle masses of the fin. These fibres show staining reactions typical of collagen and are birefringent when observed by polarized light microscopy. None of these connective tissue fibres exhibited staining reactions typical of elastin. A delicate meshwork of obliquely oriented connective tissue fibres is evident in the transverse and dorso-ventral muscles in transverse section planes (Plate IIIc). The fibres extend from the median fascia to the superficial fasciae at an angle of approximately 45° with respect to the muscle fibres in which they are embedded. The mean angle between the connective tissue and muscle fibres of the dorsal and ventral transverse muscles was measured to be 43·4° (S.D. = 6·3°) and 42·5° (S.D. = 6·1°), respectively. The mean angle for the connective tissue fibres of the dorsal and ventral dorso-ventral muscles was 45·8° (S.D. = 4·7°) and 46·3° (S.D. = 5·7°), respectively.

Connective tissue fibres that are aligned parallel to the muscle fibres are also evident in the musculature of the fin (Plate IIIc). Unlike the other connective tissue fibres that follow a more or less straight course, these fibres are kinked or folded when observed in the material prepared for light microscopy. Connective tissue is also evident at the interface between the muscles of the fin (e.g. between the transverse and dorso-ventral muscles) (Plate IIIb). Portions of this connective tissue extend into the muscles and subdivide them into smaller bundles.

The main blood vessels that supply and drain the fin tissue are located in the plane of the median fascia of the fin. These vessels are oriented with their long axes at approximate right angles to the fin cartilage and can be observed in parasagittal sections to be located at regular intervals along the length of the fin (Plate IIa). The main fin nerves are also located in the plane of the median fascia,

PLATE II. (a) Photomicrograph of parasagittal section of fin of *Sepia officinalis*. Note that the fin consists of a tightly-packed, three-dimensional array of musculature. DF, dorsal fascia; D-V, dorso-ventral muscle; L, longitudinal muscle; MF, median fascia; N, nerve; T, transverse muscle; V, vein; VF, ventral fascia. Brightfield microscopy of 7 μm polyester wax section stained with Milligan Trichrome. The scale bar length equals 1 mm. (b) Photomicrograph of transverse section of fin of *Sepia officinalis*. The section is slightly oblique to the long axis of the transverse muscle fibres (T) and the section plane therefore passes through both the dorso-ventral (D-V) and transverse muscle masses. Bundles of transverse muscle fibres can be seen to branch off from the main muscle mass (arrowheads) and extend to insert on the median fascia (MF). The letter labels are defined in caption for (a). Brightfield microscopy of 10 μm polyester wax section stained with Milligan Trichrome. The scale bar length equals 1 mm.
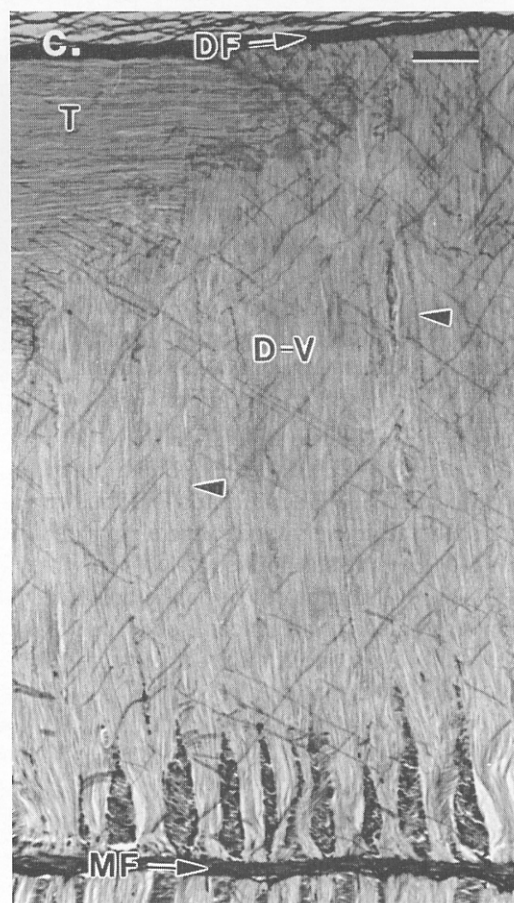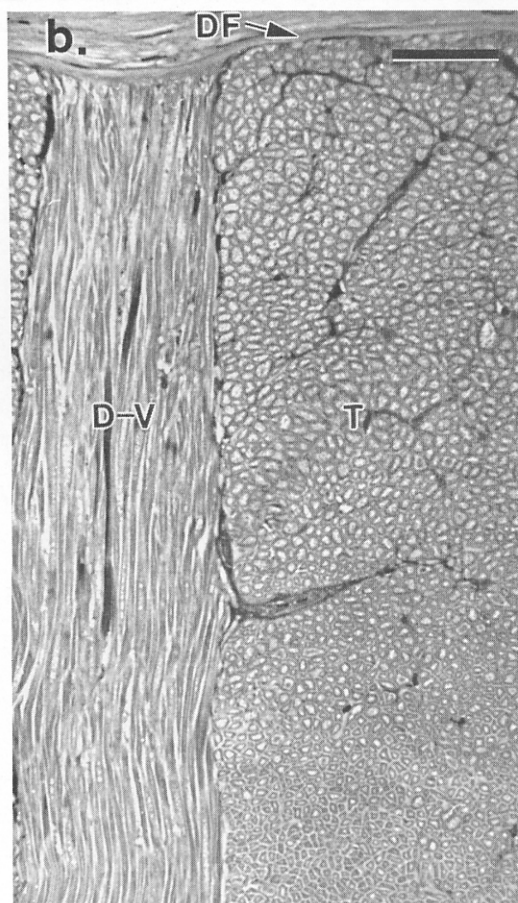
with their long axes perpendicular to the fin cartilage (Plate IIa). A range of axon diameters is observed in the fin nerves and previous work (Kier, Messenger & Miyan, 1985) suggests that both afferent and efferent axons are present in the fine nerves.

The dorsal and ventral fasciae are covered by a connective tissue dermis which is in turn covered by epithelium (Fig. 1). The epithelium is simple, cuboidal, and on the ventral surface it includes many cells with large vacuoles containing intensely staining granules (red with Milligan's Trichrome; black with Picro-Ponceau with haematoxylin). The basal lamina of the epithelium covers a thin layer of muscle fibres. The muscle fibres of this layer are arranged as an anastomosing network. A loose dermal connective tissue layer is adjacent to the anastomosing network of muscle and extends to the level of the dorsal and ventral fasciae. The connective tissue fibres of the dermal layer are arranged as a fibrous meshwork. On the dorsal surface of the fin, numerous chromatophores and iridophores are present in the dermal connective tissue layer. The dermal connective tissue layer also includes blood vessels which are located principally in the portion of the dermis immediately adjacent to the dorsal and ventral fasciae.

Ultrastructure of the fin musculature

The muscles of the fins of Sepia officinalis consist of closely packed obliquely striated muscle cells similar to those observed in a variety of muscles from cephalopods (see Kier, 1985). The myofilaments of these fusiform-shaped cells surround a central, longitudinally oriented core containing the nucleus and mitochondria (Plate IV). The presence of the mitochondrial core allows these cells to be classified as 'regular, obliquely striated' according to Millman (1967).

The mitochondrial content of the cells of the transverse muscle bundles was not uniform throughout the bundle. Instead, muscle cells of the transverse bundles in a narrow zone adjacent to the dorsal and ventral fasciae include a significantly larger core of mitochondria than the other cells of the transverse bundles and the cells of the other muscle bundles of the fin (Plates IIIb & V). Measurements from transverse sections show that the mitochondrial core of the muscle fibres adjacent to the fasciae occupies a mean of 23·4% (S.D. = 11·2%) of the fibre cross-sectional area. The mitochondrial core of the remainder of the cells of the transverse muscle bundles occupies an average of 5·9% (S.D. = 5·2%) of the fibre cross-sectional area. The mean mitochondrial area

PLATE III. (a) Photomicrograph of frontal section of fin of Sepia officinalis. The section is slightly oblique to the plane of the longitudinal muscle layers (L) and the sectional plane therefore passes sequentially (from left to right in the figure) through the transverse (T) and dorso-ventral (D-V) muscles in the dorsal half of the fin, longitudinal muscle layers (L) and then the dorso-ventral and transverse muscles in the ventral half of the fin. A nerve (N) and blood vessel (V) located in the plane of the median fascia are also included in the section. Lateral branching of a transverse muscle bundle is indicated (arrow head). Brightfield microscopy of 10 μm polyester wax section stained with Milligan Trichrome. The scale bar length equals 1 mm. (b) Photomicrograph of parasagittal section of fin of Sepioteuthis sepioidea showing transverse (T) and dorso-ventral (D-V) muscles in proximity of the dorsal fascia (DF). The transverse muscle fibres in a zone adjacent to the dorsal fascia include a more extensive core of mitochondria than those in the remainder of the transverse muscle mass (see Plate V). Dark staining connective tissue is present between the muscle masses and in addition can be observed to extend into the transverse muscle mass. Brightfield microscopy of 1·5 μm glycol methacrylate section stained with Lee's methylene blue-basic fuchsin. The scale bar length equals 50 μm. (c) Photomicrograph of transverse section of fin of Sepioteuthis sepioidea showing musculature and connective tissue in dorsal half of the fin. Note the network of connective tissue fibres oriented at approximately 45° to the long axes of the dorso-ventral muscle fibres (D-V). Kinked or folded connective tissue fibres are also present (arrow heads) and are oriented parallel to the muscle fibres. DF, dorsal fascia; MF, median fascia; T, transverse muscle fibres. Polarized light microscopy of 10 μm paraffin section stained with Milligan Trichrome. The scale bar length equals 100 μm.
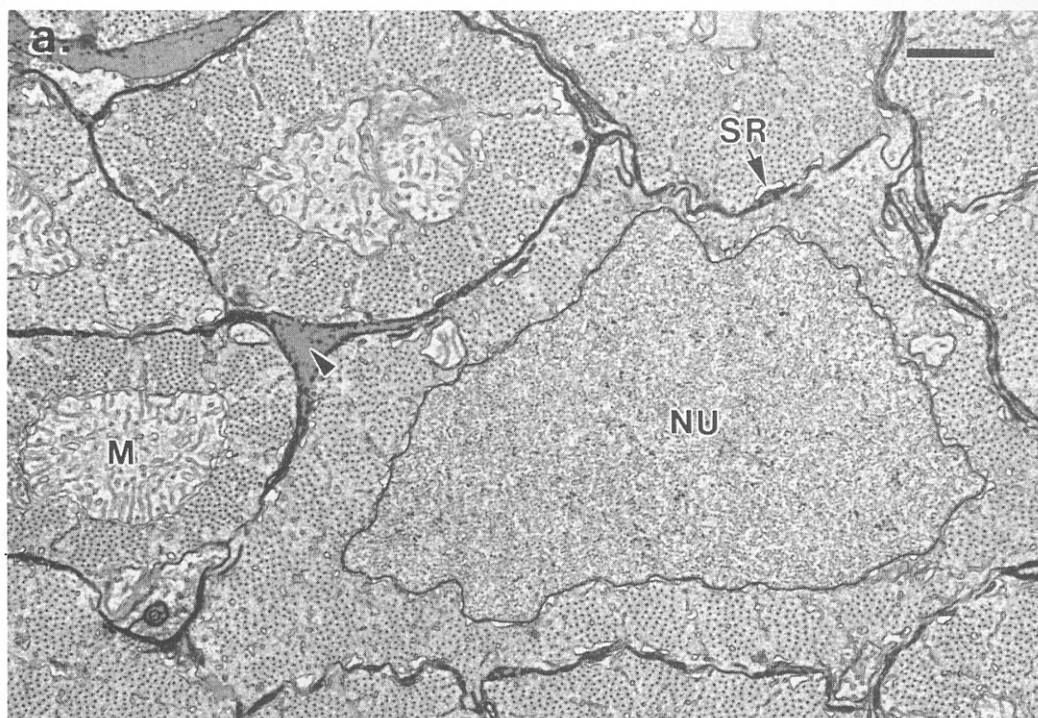
(expressed as the percentage of total cell area) of the two cell types is significantly different $(t = 6\cdot57, P \ll 0\cdot001)$. The cross-sectional area of the myofilaments of the two cell types was not different $(t = 0\cdot07, P = 0\cdot94)$. Although individual muscle cells with large mitochondrial cores were occasionally observed in the dorso-ventral and longitudinal muscle bundles, a distinct zone of such cells was observed only in the transverse muscle bundles.

The mean diameter of the cells measured from transverse sections was approximately 4 μm (mean = 3·9 μm, S.D. = 1·1 μm) for the cells with large mitochondrial cores and approximately 2 μm (mean = 1·9 μm, S.D. = 0·5 μm) for the remainder of the cells. The range of diameters observed in transverse section is probably due to the plane of section cutting through different locations along the length of these fusiform cells (see Amsellem & Nicaise, 1980; Bone *et al.*, 1981; Kier, 1985). The thick filament diameter at the middle of the thick filaments was measured to be approximately 30 nm (mean = 33 nm, S.D. = 3 nm). Thick filaments could be traced for up to 4·2 μm in longitudinal sections in which the cell remained in the sectional plane across the entire section.

Neuromuscular junctions were often observed in the musculature of the fin (Plate Vb). The nerve terminals run adjacent to the muscle cells or in a groove in the muscle cell formed by a complex infolding of the sarcolemma of the muscle cell. The terminals contain mitochondria and 50 nm (mean = 56 nm, S.D. = 9 nm) electronlucent synaptic vesicles. The opposed membranes of the junction show increased electron density. Similar neuromuscular junctions have been described in other cephalopods by Amsellem & Nicaise (1980), Bone *et al.* (1981) and Graziadei (1966).

Discussion

Biomechanics of the fin musculature

Cuttlefish and squid lack hardened skeletal support elements that extend through the fin as seen, for example, in the teleost fishes. Instead, as described above, the fins consist of a tightly packed three-dimensional array of musculature. In this regard, they resemble other 'muscular-hydrostats' (Kier & Smith, 1985) in which the musculature serves as the effector of movement and in addition provides the support for movement. The tightly-packed array of musculature of the fin constitutes a structure that is constant in volume because the muscle is essentially incompressible (Kier, 1982; Kier & Smith, 1985); there is no evidence of significant flow of fluid into or out of the fin and there are no compressible gas-filled spaces in the fin. Because the fin is constant in volume, any decrease in one dimension must result in an increase in another dimension. It is by virtue of this principle that support for movement is provided by the various muscle arrangements of the fin.

In order to examine the role of the various muscle masses of the fin in creating the undulatory waves, it is useful to consider a portion of the fin during the passage of a wave. The general form of movement of a given portion of the fin involves sequential bending dorsally and then ventrally.

PLATE IV. (a) Electron micrograph of transverse section of the obliquely striated muscle cells of the transverse muscle bundles of the fin of *Sepia officinalis*. The core of the muscle fibre is occupied by mitochondria (M) and the nucleus (NU). The cell diameter is greatest at the location of the nucleus. Note the electron dense material (arrowhead) between the cells. SR, sarcoplasmic reticulum. The scale bar length equals 1 μm. (b) Electron micrograph of longitudinal section of the obliquely striated muscle cells of the dorso-ventral muscle of the fin of *Sepia officinalis*. M, mitochondria. The scale bar length equals 1 μm.
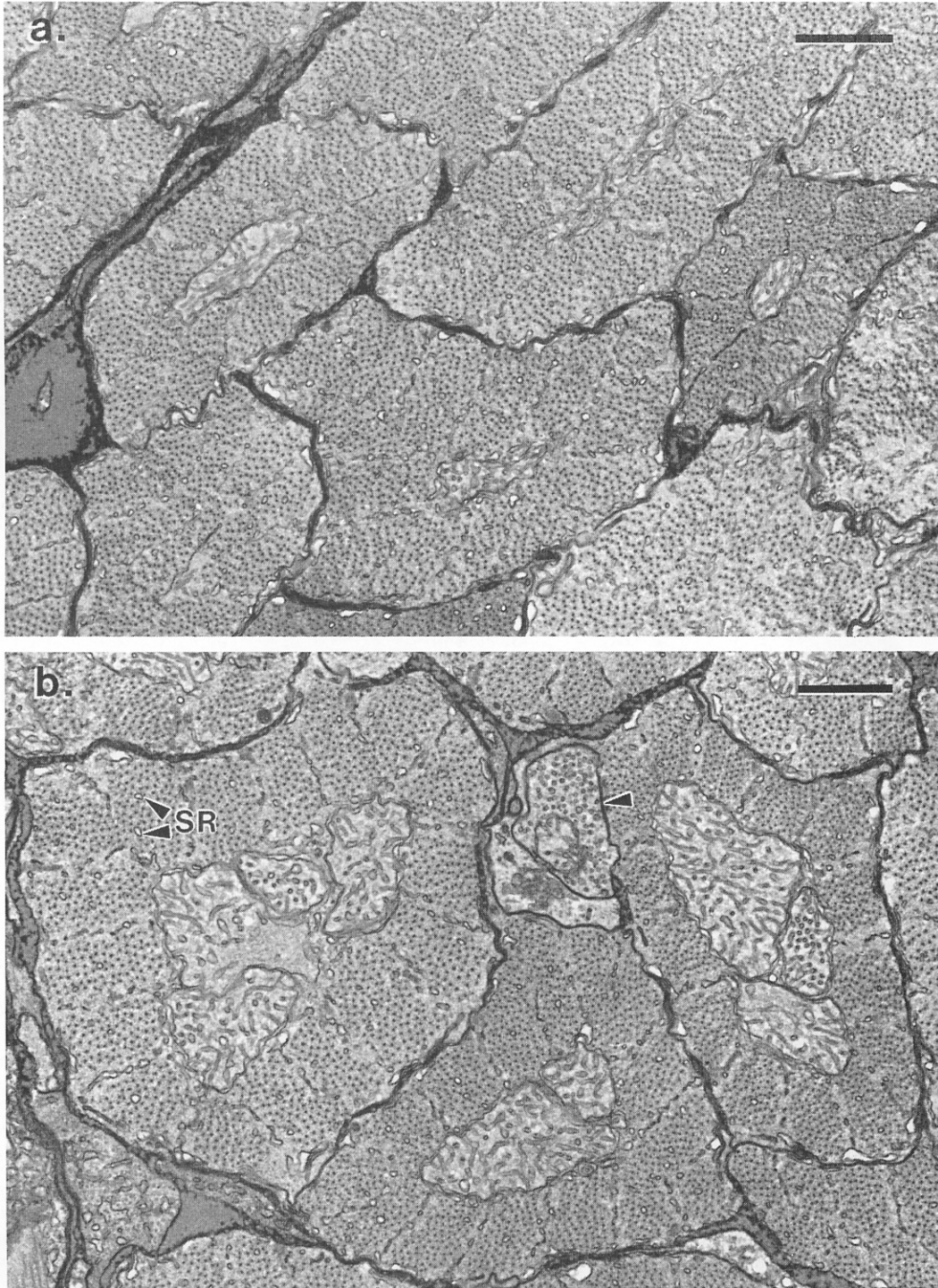
Dorsal bending requires that the musculature reduces the width of (laterally compresses) the dorsal portion of the fin relative to the ventral portion. The dorsal transverse muscle bundles are arranged such that their contraction could provide the required lateral compression of the dorsal surface. However, this contraction will cause significant bending only if the lateral compressional force on the ventral portion of the fin is resisted. Without resistance to the lateral compressional force, contraction of the dorsal transverse muscle bundles will simply pull the fin margin medially, reducing the width of the fin. Because the fin is essentially constant in volume, a decrease in width must result in either an increase in the thickness of the fin or an increase in length (or both). The dorso-ventral muscles are arranged such that they could resist increase in thickness of the fin, and the longitudinal muscles are arranged such that they could resist increase in length of the fin. Thus, without the contribution of some other component of the fin, bending requires simultaneous contractile activity of all three muscle orientations.

The above discussion implies that the dorso-ventral muscle is responsible for simply maintaining the thickness of the fin during bending. However, a more active role in bending the fin can be envisioned for the dorso-ventral muscles. For instance, bending of the fin dorsally could also be produced by lateral extension of the ventral portion of the fin relative to the dorsal surface. Lateral extension of the ventral portion could be caused by contraction of the ventral dorso-ventral muscles. Contractile activity of the dorsal transverse muscle bundles would then be required to prevent lateral extension of the dorsal surface of the fin and longitudinal muscle activity would be required to prevent longitudinal extension. In fact, these two conditions (transverse muscle shortening with dorso-ventral muscle activity and dorso-ventral muscle shortening with transverse muscle activity) represent end points in a continuum of relative contraction of the transverse and dorso-ventral muscle bundles. Simultaneous shortening of the dorsal transverse muscle bundles and ventral dorso-ventral muscle bundles would create a more pronounced bend than shortening of one of the orientations with the other simply maintaining constant length by contractile activity.

The possible role of the various muscle arrangements of the fin in producing the undulatory waves of the fin is as follows. Dorsal bending of the fin probably involves simultaneous contractile activity in the dorsal transverse muscle bundles and the ventral dorso-ventral muscle bundles. Ventral bending of the fins requires simultaneous contractile activity in ventral transverse muscles and dorsal dorso-ventral muscles. In addition, these movements must be accompanied by contractile activity in the longitudinal muscles, thereby providing control of the longitudinal dimension of the fin. It might be argued that the role of the longitudinal muscle could be more economically served by longitudinally oriented connective tissue fibres. However, the amplitude of the undulatory waves is variable and therefore the length of the fin at its edge must also be variable. The length of the fin at its edge is the least when the fins are extended laterally without undulatory waves, for example, during the dymantic response (Boycott, 1958). It is greatest during vigorous fin beating when large amplitude undulatory waves pass down the fin. Control of the length of the fin and the amplitude of the undulatory waves could be provided in part by the longitudinal muscle.

PLATE V. Electronmicrographs at same magnification of transverse sections of the obliquely striated muscle cells of the transverse muscle bundles of the fin of *Sepia officinalis*. The cells in (b) are found in the zone adjacent to the fascia of the fin (see Plate IIIb) and show a more extensive mitochondrial core than the remainder of the cells of the transverse muscle mass as shown in (a). A neuromuscular junction (arrow head) is shown in (b). Note that the tubules of the sarcoplasmic reticulum (SR) extend into the myofilament mass. The scale bar length equals 1 μm.

The role of the oxidative and anaerobic glycolytic muscle fibres

Investigations of the ultrastructure of the musculature revealed two zones within the transverse muscle fibres. The fibres in these two zones differ in mitochondrial content and resemble the analogues of red and white muscle that have been observed in the mantle musculature of squid and cuttlefish (Bone *et al.*, 1981; Mommsen *et al.*, 1981). In this work on the mantle musculature, analysis of enzymatic activity and histochemistry has shown that the mantle muscle fibres with a more extensive mitochondrial core are specialized for oxidative metabolism, whereas the fibres with a less extensive mitochondrial core primarily use anaerobic glycolysis. It is likely that the two fibre types in the transverse muscle of the fins serve different functional roles, as in the mantle. If so, the transverse muscle fibres with a more extensive mitochondrial core (the oxidative fibres) are responsible for creating the almost constant, low amplitude undulatory waves that are observed when the animal is at rest on the bottom or slowly hovering. The brief bursts of large amplitude waves observed during prey capture and rapid manoeuvring and locomotion are probably created by the transverse muscle fibres with less extensive mitochondrial core (the anaerobic glycolytic fibres).

The arrangement of the two fibre types in the fin, with the layer of superficial oxidative transverse fibres enclosing the anaerobic glycolytic transverse fibres, has two possible mechanical consequences. First, the oxidative muscle fibres are situated further from the neutral axis of the fin and thus have a greater moment available for fin bending than if they were located closer to the median fascia. This arrangement is reminiscent of that seen in the locomotor muscle of bony fishes (Bone, 1978*a, b*). Secondly, the anaerobic glycolytic muscle, although not active during the low amplitude waves, could serve as the hydrostatic fluid enclosed by the container of the active oxidative muscle layer (Mommsen *et al.*, 1981).

The role of the crossed connective tissue fibres

The muscle masses of the fin other than the transverse muscles lack zones of muscle fibres with extensive mitochondrial cores. The above discussion of fin movement hypothesizes that these other muscle masses also provide the support required for the bending fin movements and thus, it would be expected that they should include fibres that have a similar oxidative capacity to that of the oxidative muscle fibres of the transverse muscle. The lack of such fibres implies that some other component of the fin provides the resistance to lateral compression caused by contraction of the oxidative transverse muscle fibres during gentle fin beating. Mechanical analysis indicates that the obliquely oriented connective tissue fibres of the fin could provide this support. The connective tissue fibres in the transverse and dorso-ventral muscle masses are oriented at an angle of 45° relative to the long axes of the muscle fibres. In a block of muscle fibres of constant volume with connective tissue fibres oriented at an angle of 45° to the long axes of the muscle fibres, shortening or lengthening of the muscle fibres of the block will increase the length of these obliquely-oriented connective tissue fibres (as long as the third dimension is held constant). Lateral compression of the fin caused by contraction of the dorsal and ventral oxidative muscle fibres would be resisted because these presumably stiff connective tissue fibres would be placed in tension and would thereby limit the deformation of the fin during gentle fin beating. The elastin-like fibres observed in the median fascia may play a role in controlling the length of the fin during these movements. Thus, oxidative muscle fibres in the dorso-ventral and longitudinal muscles are not required during gentle fin beating because the support may be provided by the crossed connective tissue fibres. During vigorous fin beating, it is likely that muscles of all three orientations are active.

Connective tissue fibres oriented obliquely to muscle fibres have been described previously in the mantle musculature of squid and cuttlefish (Bone *et al.*, 1981; Gosline & Shadwick, 1983*a*, *b*; Gosline *et al.*, 1983). These fibres, termed 'intermuscular fibres' by Gosline & Shadwick (1983*a*), appear to serve as a system for elastic energy storage that provides for muscular antagonism and may increase the efficiency of the mantle musculature, improving locomotor performance (Gosline & Shadwick, 1983*a*, *b*). Although further research on the mechanical properties of the fin tissue is required, it is possible that the obliquely arrayed connective tissue fibres of the fin described here could also serve in elastic energy storage during fin beating, improving the efficiency of the fin musculature.

Summary

The fins of cuttlefish and squid are examples of 'muscular-hydrostats', structures that lack the characteristics of the two general categories of skeletal support found in animals; the fins lack the extensive fluid-filled cavities that characterize most hydrostatic skeletons and no hardened, rigid internal or external skeletal elements are present. The fins consist primarily of a tightly-packed, three-dimensional array of musculature. This musculature serves both to create force for movement and to provide the skeletal support necessary for movement. This dual role for the musculature is possible because the fin is constant in volume and the musculature is arranged so that deformation of the fin can be controlled in three mutually perpendicular directions.

Two features of mechanical significance were observed in the fins that were not previously noted in muscular hydrostats, such as the arms and tentacles of cephalopods. The first is a possible functional division of muscle fibres based on aerobic capacity. The fin includes zones of obliquely striated muscle fibres that may possess a greater aerobic capacity than the remainder of the fin musculature. It is hypothesized that these oxidative muscle fibres create the gentle fin movements seen when the animals are hovering or resting. The second feature concerns the arrangement of connective tissue fibres in the musculature. This network of connective tissue fibres is oriented at an angle of approximately 45° to the long axes of the muscle fibres and could provide the support required for the gentle fin movements. The short bursts of vigorous fin beating seen during prey capture and rapid manoeuvring probably involve the anaerobic glycolytic obliquely striated muscle fibres that constitute most of the fin musculature. The network of connective tissue fibres may provide a mechanism for elastic energy storage during fin beating.

REFERENCES

Amsellem, J. & Nicaise, G. (1980). Ultrastructural study of muscle cells and their connections in the digestive tract of *Sepia officinalis*. *J. submicrosc. Cytol.* **12**: 219–231.

Bennett, H. S., Wyrick, A. D., Lee, S. W. & McNeil, J. H. (1976). Science and art in preparing tissues embedded in plastic for light microscopy, with special reference to glycol methacrylate, glass knives and simple stains. *Stain Technol.* **51**: 71–97.

Bidder, A. & Boycott, B. B. (1956). Pelagic Mollusca: swimmers and drifters. *Nature, Lond.* **177:** 1023–1025.

Bone, Q. (1972). Some notes on histological methods for peripheral nerves. *Med. Lab. Technol.* **29:** 319–324.

Bone, Q. (1978a). Locomotor muscle. In *Fish physiology* **7:** 361–424. Randall, D. J. & Hoar, W. S. (Eds). New York: Academic Press.

Bone, Q. (1978b). Myotomal muscle fiber types in *Scomber* and *Katsuwonus*. In *The physiological ecology of tunas*: 183–205. Sharp, G. D. & Dizon, A. E. (Eds). New York: Academic Press.

Bone, Q., Pulsford, A. & Chubb, A. D. (1981). Squid mantle muscle. *J. mar. Biol. ass. U.K.* **61:** 327–342.

Boycott, B. B. (1958). The cuttlefish—*Sepia. New Biol.* **25:** 98–118.

Gosline, J. M. & Shadwick, R. E. (1983a). The role of elastic energy storage mechanisms in swimming: an analysis of mantle elasticity in escape jetting in the squid, *Loligo opalescens. Can. J. Zool.* **61:** 1421–1431.

Gosline, J. M. & Shadwick, R. E. (1983b). Molluscan collagen and its mechanical organization in squid mantle. In *The Mollusca* **1:** 371–398. Hochachka, P. W. (Ed.). New York: Academic Press.

Gosline, J. M., Steeves, J. D., Harman, A. D. & Demont, M. E. (1983). Patterns of circular and radial muscle activity in respiration and jetting of the squid *Loligo opalescens. J. exp. Biol.* **104:** 97–109.

Graziadei, P. (1966). The ultrastructure of the motor nerve endings in the muscles of cephalopods. *J. Ultrastruct. Res.* **15:** 1–13.

Gurr, E. (1956). *A practical manual of medical and biological staining techniques.* New York: Interscience.

Humason, G. L. (1979). *Animal tissue techniques.* 4th edn. San Francisco: W. H. Freeman & Co.

Kier, W. M. (1982). The functional morphology of the musculature of squid (Loliginidae) arms and tentacles. *J. Morph.* **172:** 179–192.

Kier, W. M. (1985). The musculature of squid arms and tentacles: ultrastructural evidence for functional differences. *J. Morph.* **185:** 223–239.

Kier, W. M. (1987). The functional morphology of the musculature of the tentacles of *Nautilus*. In *Nautilus: biology and paleobiology of the living fossil*. Saunders, W. B. & Landman, N. H. (Eds). New York: Plenum Press.

Kier, W. M. (1988). The arrangement and function of molluscan muscle. In *The Mollusca, form and function* **11:** 211–252. Trueman, E. R., & Clarke, M. R. (Eds). New York: Academic Press.

Kier, W. M. & Smith, K. K. (1985). Tongues, tentacles and trunks: the biomechanics of movement in muscular-hydrostats. *Zool. J. Linn. Soc.* **83:** 307–324.

Kier, W. M., Messenger, J. B. & Miyan, J. A. (1985). Mechanoreceptors in the fins of the cuttlefish, *Sepia officinalis. J. exp. Biol.* **119:** 369–373.

Mallory, F. B. (1944). *Pathological technique.* Philadelphia: W. B. Saunders.

Messenger, J. B., Nixon, M. & Ryan, K. P. (1985). Magnesium chloride as an anaesthetic for cephalopods. *Comp. Biochem. Physiol. (C)* **82:** 203–205.

Milligan, M. (1946). Trichrome stain for formalin-fixed tissue. *Am J. Clin. Pathol. Tech. Sect.* **10:** 184–185.

Millman, B. M. (1967). Mechanism of contraction in molluscan muscle. *Am. Zool.* **7:** 583–591.

Mommsen, T. P., Ballantyne, J., MacDonald, D., Gosline, J. M. & Hochachka, P. W. (1981). Analogues of red and white muscle in squid mantle. *Proc. natn. Acad. Sci. U.S.A.* **78:** 3274–3278.

Packard, A. & Trueman, E. R. (1974). Muscular activity of the mantle of *Sepia* and *Loligo* (Cephalopoda) during respiratory movements and jetting, and its physiological interpretation. *J. exp. Biol.* **61:** 411–419.

Palmgren, A. (1948). A rapid method for selective silver staining of nerve fibres and nerve endings in mounted paraffin sections. *Acta zool., Stockh.* **29:** 377–392.

Palmgren, A. (1955). Staining nerve fibres after sublimate-acetic and after Bouin's fluid. *Stain Technol.* **30:** 31–36.

Palmgren, A. (1960). Specific silver staining of nerve fibres. 1. Technique for vertebrates. *Acta zool., Stockh.* **41:** 239–265.

Reynolds, E. S. (1963). The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *J. Cell Biol.* **17:** 208–212.

Russell, F. S. & Steven, G. A. (1930). The swimming of cuttlefish. *Nature, Lond.* **125:** 893.

Steedman, H. F. (1957). Polyester wax. A new embedding medium for histology. *Nature, Lond.* **179:** 1345.

Tompsett, D. H. (1939). *Sepia. L. M. B. C. Mem.* **32:** 1–184.

Tittel, K. (1961). Der funktionelle Aufbau des Tintenfischarmes im Vergleich mit dem Muskelkörper der Säugerzungen. *Verh. anat. Ges., Jena* **57:** 264–275.

Tittel, K. (1964). Saugnapf-, epi- und hypofasciale Armmuskulatur der Kephalopoden-ein Beitrag zur funktionellen Anatomie freibeweglicher Skelettmuskelkörper. *Gegenbaurs morph. Jb.* **106:** 90–115.

Ward, D. V. (1972). Locomotory function of the squid mantle. *J. Zool., Lond.* **167:** 487–499.

Ward, D. V. & Wainwright, S. A. (1972). Locomotory aspects of squid mantle structure. *J. Zool., Lond.* **167:** 437–449.