REVIEW

Gelatinous animals and physiology

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This brief review discusses the different ways in which the study of gelatinous plankton has contributed to an understanding of the physiology and evolutionary relationships of other animals.

INTRODUCTION

This review is mainly concerned with experimental work on all kinds of gelatinous plankton, that has had a direct or indirect influence on such general problems as nervous transmission or membrane ion channels. The large corpus of important and distinguished work using echinoderm larvae as models to study developmental processes (including the cell cycle) will not be considered here. Nor will the classic work on squid giant axons, for although gelatinous, the small planktonic cephalopods do not have sufficiently large giant axons for experiment. It might seem curious that work on jellyfish or chaetognaths could yield any insights to give to physiologists working on 'higher' animals. Yet physiological observations on the gelatinous plankton began early, and Charles Darwin was one of the first to make them, when he collected salps on H.M.S. Beagle, remarking in January 1832, on their regular pulsation (19 beats in 30 s) and on the rapid circulation of the blood in the gill bar (Keynes, 2000).

Almost all animals of the gelatinous plankton are delicate, difficult to maintain in the laboratory, and only available at certain favoured marine laboratories. Despite this constraint, physiological work on medusae of different kinds, or on pelagic tunicates, as well as being of much intrinsic interest, has had implications for physiology in general, in particular for cell and neuromuscular physiology and medicine. There are of course a few neritic species which are exceptions to the rule that all gelatinous plankton are difficult to keep for experiment. Some, like the scyphozoan jellyfish *Cassiopea xamachana*, which lives near mangroves, or the small chaetognath *Sagitta hispida* are relatively robust and easy to maintain alive for experiment.

Study of several of the most fascinating and challenging physiological problems faced by gelatinous plankton has not (as yet) contributed much to general physiology. For instance, many animals of the gelatinous plankton are extremely, even glassily, transparent, some to such an extent that it is nearly impossible to see them even in a small jar held up to the light. All that can be seen of some Alciopid polychaetes under these conditions are a pair of crimson eyes and a single row of black spots apparently swimming around independently without any corporeal link. Intriguing as it is, transparency of gelatinous plankton has yet to yield any insights for physiology in general, although this perhaps may change with further studies, see Johnsen (2001).

Similarly, although buoyancy and its control are of supreme importance to members of the gelatinous plankton, and have been a good deal studied, this has not had much influence on other fields of physiology. Fish buoyancy has engaged the attention of physiologists of the highest calibre (see Denton, 1961), and the only 'invention' of the gelatinous plankton not seen in fish is the storage of ammonium ions to provide static lift in several groups (chaetognaths, cephalopods and probably pelagic tunicates). Indeed, their only relevance to general physiology and biochemistry is that they have provided examples such as salps or chaetognaths where simple structures may resolve such problems as the diversity and organization of 'simple' ion pumps, or the origins of NH_4^+ transfer across membranes.

Those gelatinous plankton whose buoyancy maintains them in the upper photic zone, however, may possibly contain UV screening pigments useful for commercial synthesis and use.

Naturally, most physiologists work on vertebrates, so that some of the discoveries made on gelatinous animals came as a considerable surprise. For example, the report by Mackie & Meech (1985) that a single axon could propagate two types of action potentials was entirely unexpected. Again, luminescence in the hydrozoan jellyfish *Aequorea* was thought to rely on a luciferase reaction, as in fireflies, and it came as a real

surprise to find that a quite different luminescent system was involved (see Shimomura, 1995).

Many different groups have gelatinous representatives in the plankton, either at all stages, or only as larvae. Amongst metazoa, larval and adult Cnidaria predominate in numbers of species and perhaps also in absolute numbers, but there are also many others: annelids, molluscs, chaetognaths, echinoderms, hemichordates, pelagic tunicates, and perhaps surprisingly, adults of some primarily benthic groups, such as holothuria. Local blooms of gelatinous plankton like thaliaceans may contain remarkable numbers, covering the sea for many square miles. Chaetognaths may even be so abundant as to turn the sea greyish. Larval forms of most phyla are planktonic, and although few have been material for physiologists, some have attracted interest, for example, molluscan veligers for ciliary control (Mackie et al., 1976; Arkett et al., 1987). Most studies have been on echinoderm larvae, classical material for developmental physiology, but also, more recently, those favourites of speculative zoologists, the sessile tunicate tadpole larvae and the amphioxus larva. These latter have naturally been of much interest to exponents of evo-devo rather than to physiologists directly, but the tunicate tadpole certainly has attracted physiologists interested in the control of oscillatory swimming (e.g. McHenry & Patek, 2004; Brown et al., 2005).

Most gelatinous animals share such features as transparency (nerves and muscle fibres are often visible in the living animal), and many are an appropriate size for experiment, though usually also sharing the disadvantages of only being readily obtainable at a few marine laboratories and being difficult to maintain alive. Even if apparently in good condition, it is necessary to be aware that captive specimens in the laboratory may behave very differently to those observed in the sea (by divers for example, see Harbison & Gilmer, 1992).

Two aims have usually driven physiologists to work with gelatinous animals: first the search for 'new' preparations to reveal general principles of function, and secondly, the attempt to deduce from 'simple' forms the steps whereby the more complex systems of 'higher' groups may have evolved. An example of the former is provided by that curious and isolated phylum the chaetognaths, where recent work (Jean et al., 2004) has shown that the group can provide useful models for the study of heat shock proteins. For the second instance, voltage-gated ion channels in cnidaria and other 'lower' animals have attracted interest in the hope that comparison of different channel versions may provide useful information about ion channel structure and function (see Anderson &

Greenberg, 2001). Another example in which metazoan phylogeny may benefit from work on gelatinous plankton is the recent interest in the origin of mesoderm and its possible equivalent in medusae, together with the presence of such genes as MyOD in cnidarians (Müller et al., 2003).

However, on the whole, most physiologists working with gelatinous plankton have been interested in muscular systems and their nervous control, in sensory capacities and in the mode of action of toxins produced by dangerous medusae. Fewer workers have studied topics such as transparency, buoyancy, digestion, or feeding, and these topics have as yet contributed little to general physiology.

1. NERVOUS SYSTEMS

The majority of the first physiologists who worked with gelatinous plankton became interested in the locomotion of medusae and how it was controlled. Their work had a considerable influence on early neuromuscular physiology and on the understanding of the general operation of the nervous system itself. The classic work of Romanes (1876, 1877, 1880), (aided by the studies on the nervous system and sense organs by those remarkable histologists the Hertwig brothers (1878), and a little later by Schäfer's (1879) work on the medusa nerve net), began what after the last war became a very fruitful field of neuromuscular physiology. The development of new methods, including polyethylene suction electrodes, intracellular recording, dye injection and antisera, patch-clamping, electron microscopy and sensitive tension recording all contributed to a renaissance in work on gelatinous plankton. Influenced by Passano and Mackie, several younger neurophysiologists in North America studied the nervous systems and control of locomotion in hydromedusae and pteropods. Although these more recent studies were of very considerable interest in demonstrating the capabilities of the medusan nervous system in controlling locomotion, few had the same aim (or hope) of adding to the general knowledge of the nervous system as had Romanes and other earlier workers.

Nevertheless, the work of Mackie and Meech on the dual action potential system and the neural circuitry of the small hydrozoan jellyfish *Aglantha* (summarized by Mackie, 2004) did much interest neurophysiologists of 'higher' forms.

At the beginning of the 20th Century, as Passano (1982) pointed out, 'physiologists had no convenient nerve preparation beyond the familiar frog sciatic nerve/gastrocnemius muscle'. Romanes' (1876, 1877, 1880) work on hydrozoan and scyphozoan medusae,

together with later work on the entrapped wave preparation in the scyphozoan medusa *Cassiopea* (Mayer, 1906) had seemed to be a hopeful way in which such properties of the nerve impulse as conduction velocity, temperature sensitivity and ionic requirements could be discerned. Unfortunately, this hope was not to be realized, as the histological basis for the conduction wave was a large number of neurons connected in an unknown way, rather than a parallel line up of axons. Nevertheless, with simple experiments, both Romanes (1877) and Mayer (1916) were able to give accurate measures of conduction velocity across a nerve net (Romanes) and along an isolated *Cassiopea* ring (Mayer). More recently, these have been updated in a review by Satterlie (2002), whilst Mackie (2004) himself has reviewed his long series of studies with his colleagues on the exceptional hydrozoan medusa *Aglantha* in which he recognized no fewer than 14 separate conducting systems in the nerve rings. The reader is therefore referred to these two latter reviews for details of the systems, and only a short general overview will be attempted here, what Romanes (1880) describing the Hertwigs' work, called 'an epitome of an epitomy'.

The series of papers in which Romanes (1876, 1880, 1895), described his early experimental work on hydromedusae and the scyphozoan medusa *Aurelia* carried, as Mackie (1980), justly remarked, 'such an aura of finality that it is hardly surprising that few other workers ventured into the field until quite recently'. Among Romanes' many discoveries were the dichotomy between the hydromedusan and scyphozoan nervous systems (also noted by Eimer, 1877), facilitation and conduction velocity.

In a couple of decades after Romanes pioneering work, the scyphomedusan specialist Mayer took up work across the Atlantic on a different scyphomedusan, *Cassiopea*. This medusa turned out to be exceptionally hardy, and could be kept for many days in the laboratory even withstanding changes in temperature, pH, and up to 42 days starvation! Mayer studied several aspects of the growth and regeneration of *Cassiopea* but his best known work was upon rates of nerve conduction and the mechanism of nerve conduction Mayer (1916). By altering the external solution for strips of the medusa including an eye, he showed that nerve conduction (and muscular contraction) required Na^+, Ca^{2+} and K^+. Whilst Mayer's theory of nerve conduction (involving adsorption of these ions onto negatively charged colloid particles or proteids) has been superseded, his work reintroduced physiologists to medusae as experimental animals. He also followed Romanes' study of rhythmic behaviour, showing that if a smaller individual was grafted onto a larger, the more rapid rhythm of the smaller overcame that of the larger.

Excellent reviews of the very different organization and properties of the neuromuscular systems of hydrozoan, scyphozoan and cubozoan medusae have been given by Spencer & Schwab (1982), Passano (1982) and by Satterlie (2002). The many references in the text of Satterlie's review, and the title of the earlier review 'Pacemakers and activity patterns in medusae: homage to Romanes' by Passano (1965), are eloquent testimony to the excellence of Romanes' work. Suffice it to say here that Scyphozoa have no organized nerve ring like hydrozoan medusae, nor do they possess gap junctions acting as through conducting pathways, as hydrozoans do, hence epithelial action potentials are absent. There is, however, a system of small neurons, interacting with the larger marginal neurons (Passano, 2004), these seem not to have an equivalent in hydrozoans. The structure and activities of a very different coelenterate type, ctenophores, have been described by Tamm (1982). More recently, Moss & Tamm (1987) have demonstrated that a Ca^{2+} regenerative potential controlling ciliary reversal is propagated along the cilia of ctenophore comb plates, but so far as I am aware this interesting finding has not influenced studies of ciliary control in bilaterians.

2. SYNAPSES AND NEUROTRANSMITTERS

As Satterlie (2002) pointed out, Romanes was quite clear that neurons of the jellyfish nerve net were separate from each other and that there was a 'process of physiological induction' connecting them. This remarkable anticipation of synaptic transmission in medusae (at a time when the neuron doctrine was still debated) was not to be directly confirmed until Horridge & Mackay (1962) observed scyphozoan synapses by electron microscopy, as later did Mackie & Singla (1965) in hydrozoans. Spencer (1982) discussed the physiology of neuromuscular synapses in the hydrozoan *Polyorchis*, later joining with Anderson (Anderson & Spencer, 1989) to emphasize the importance of cnidarian synapses for various aspects of chemical synaptic transmission. In certain species, neurons and synapses are accessible for intracellular and voltage-clamp recordings, and have been shown to be bi-directional. However, if the structure and basic physiology of cnidarian synapses was understood, the same could not be said of the neurotransmitters employed at these synapses. As yet a family of neuropeptides terminating in a NH_2 group, (e.g. FRMF NH_2) has been recognized as a neurotransmitter and is seen immunocytochemically in many neu-

rons (first by Grimmelikhuijkzen & Spencer, 1984; see review by Grimmelikhuijkzen et al., 2002). A different possible transmitter is the amino acid taurine, found by immunocytochemistry in the ectodermal and endodermal nerve nets of the scyphozoan *Cyanea capillata* (Carlberg et al., 1995). Anderson & Trapido-Rosenthal (1990) had previously provided some electrophysiogical evidence for a taurine-like transmitter at an excitatory synapse in *Cyanea*. Carlberg and his colleagues found that taurine was co-localized with FRMF NH$_2$ in some of the diffuse endodermal net neurons.

To be established *definitively* as a neurotransmitter, rather stringent criteria have to be satisfied, and although there is good evidence for nitrergic (Moroz et al., 2004) and dopaminergic (Chung et al., 1989) transmitters in jellyfish, not all criteria have been met. As Anderson (2004) remarked, the importance of work on the cnidarian nervous system lies in the important position of the group in metazoan evolution and the evolution of the bilaterian nervous system. Merely collecting new transmitters (or indeed any other data) from cnidaria is of little value unless it relates to these topics (see section 9 on Evo-Devo), or is of importance in understanding general physiological concepts.

3. LUMINESCENCE AND PHOTOPROTEINS

Almost certainly the greatest impact (by far) that work on gelatinous plankton has made on physiology (and many other disciplines such as biochemistry, cell science and medicine) was that of the discovery, extraction and modification of two photoproteins from the jellyfish *Aequoria*: aequorin and green fluorescent protein (GFP). Aequorin emits blue light in the presence of Ca^{2+} by the oxidation of the luminophore coelenterazine. The associated small photoprotein GFP (238 amino-acids) is excited by blue light, and emits green light, which is presumably utilized by *Aequorea* itself to deter predators.

After a long series of extractions and purifications which began in 1961, amusingly described by Shimomura (1995), aequorin and other hydrozoan photoproteins are now in general use for a considerable variety of purposes, as well as the initial determination of low levels of Ca^{2+} in cells. Shimomura et al. (1963) first suggested the use of aequorin as a Ca^{2+} indicator, and it was used for this purpose in giant barnacle muscle fibres by Ridgeway & Ashley (1967). Ashley had been looking at Ca^{2+} levels in the large muscle fibres of spider crabs (*Maia squinado*) at Plymouth, working with P.C. Caldwell. In 1965, he visited Graham Hoyle's laboratory at Eugene in

Oregon and joined with Ridgeway to use the much larger and more robust fibres from *Balanus nubilis*. They spent a frustrating period using the Ca^{2+} indicator murexide without success, though gaining experience in this kind of optical experiment. Then looking in the literature, they were astounded to find Shimomura's paper in *Science* (Shimomura et al., 1963) suggesting the use of aequorin as a Ca^{2+} indicator in biological fluids. The Friday Harbor laboratory (FHL) of the University of Washington where Shimomura extracted aequorin was, as Ashley remarked, literally on the doorstep to Eugene as an added bonus! So began Ridgeway and Ashley's classic work on Ca^{2+} levels in muscle. I am indebted to Professor Christopher Ashley for this account.

In the late 1960s *Aequorea* was abundant around the docks at FHL, but no longer. The difficulty of collection of *Aequorea* led to Campbell's very successful assault on another luminescent hydrozoan, the small hydroid *Obelia geniculata* and the extraction, purification and eventual use of the photoprotein Obelin in many medical applications (Campbell, 1974).

Modification of the photoprotein GFP permits not only greater sensitivity for Ca^{2+} determination, (e.g. Shimomura & Shimomura, 1985) but also use for determining other elements, such as Zn; as a reporter of gene expression; an indicator for protein trafficking and protein localization. Modifications have also resulted in emission of different colours, as in cyanGFP, yellow GFP etc. so that it is possible to examine the expression of several proteins (for example) in a single preparation. It is now used as a safe marker for transgenic plants (Haseloff et al., 2004). Recently, GFP was sent to the Soyuz space station in an experiment to determine DNA damage in yeast cells Knight (2004).

4. LOCOMOTION

Of the different modes of locomotion used by the gelatinous plankton, both jet propulsion and undulatory propulsion are of general interest, for it is possible to find organisms of the appropriate dimensions and swimming velocity to examine an interesting and otherwise rather inaccessible range of Reynolds number (Re). Curiously, interest may not only be hydrodynamical, for the future development of minute drone aeroplanes, may make studies of jet propulsion at low Re in gelatinous plankton of some military interest! Today, so far as I am aware, medusan, tunicate and squid jet propulsion or chaetognath and ascidian larval oscillatory propulsion operate at very much lower Reynold's numbers than that of present aircraft and underwater vehicles.

The physiology of oscillatory swimming has been much studied by physiologists in different kinds of fish, like lampreys (e.g. Grillner et al. (1991)), or dogfish (Roberts, 1988). Although much progress has been made, the spinal cords of lampreys and other fish contain large numbers of neurons, and the ascidian tadpole larva offers a very simple (if not yet fully understood) neuronal network involving only a very few neurons to generate oscillatory locomotion. Here, the minimum requirements (e.g. crossed inhibition or sensory input) for oscillatory swimming may be tested. Recent studies on *Ciona* larvae (Brown et al., 2005) have shown that GABA seems to play a role in the control of swimming similar to that in higher forms. However, there are what appear to be curiously unnecessary complications in the caudal musculature of tadpole larvae where all muscle cells are electrically linked and two motoneurons supply some of them.

Possibly closer to vertebrate oscillatory locomotion may be the control system of oikopleurid larvaceans where little is known of connections within the caudal ganglion (controlling tail oscillation) to know how the system operates. Once again, unfortunately not enough is known about the chaetognath nervous system to understand how forward swimming or the fast-start reaction (Jordan, 1992) are controlled.

Most medusae swim relatively slowly, (although the small hydrozoan *Aglantha* achieves remarkable instantaneous velocities up to 50 cm s^{-1} with maximum accelerations up to 7.8 m s^{-2}. In comparison the rocket-shaped diphyid siphonophore *Chelophyes* is slower: instantaneous velocities are up to 30 cm s^{-1} and acceleration up to 5.35 m s^{-2}. There is an important difference between the jet propulsion of these two rapidly swimming animals. The first jet pulse of fast swimming in *Aglantha* is the most effective in driving it forwards, because subsequent pulses occur before refilling is complete and so it moves forward only 40–60% of the distance covered after the first pulse. Meech (2004) has discussed slow swimming in *Aglantha*, where the first jet pulse is weaker than those following, presumably to permit the jellyfish to turn before swimming upwards. In *Chelophys* rapid jetting, the first pulse is the least effective, and the most effective contraction occurs after the 5th and succeeding pulses. A possible explanation of this unexpected situation is that the first few pulses in a burst of jet pulses are weaker than those following to permit the elongate extended fishing stem of the siphonophore to be withdrawn before maximum velocities are attained, whilst the tentacles of *Aglantha* are not so fragile. Both in slow swimming by *Aglantha* and rapid swimming by *Chelophys*, the voltage-regulated channels of the myo-epithelial cells change during activity and so the contractile response is modified.

The mechanics of medusan jet propulsion were early studied by Gladfelter (1973), and by Daniel (1983, 1985). More recently there has been a revival of interest in the subject, and discussion and refinement of the models (e.g. taking account of vortex ring formation and changes in velar aperture) for both oblate and prolate medusae (see De Mont & Gosline (1988a,b,c); Colin & Costello (2002); Dabiri & Gharib (2003) and McHenry & Jed (2003)). Jet propulsion in salps, doliolids and a diphyid siphonophore has been examined by Bone & Trueman (1982, 1983, 1984).

5. EPITHELIAL CONDUCTION

Although epithelial conduction across nerve free epithelia might well be regarded as an hydrozoan and tunicate speciality, it occurs also in the young stages of amphibia and lungfish and in some respects is more easily studied and analysed in the gelatinous forms than in these vertebrates. Moreover, the activity of the stretch receptor channels in excitable epithelia is more easily accessed than, for example, in the vertebrate neuromuscular spindle. The early suggestions that neuroid conduction occurred in medusae were reviewed by Mackie (1970), who was largely responsible for the gradual acceptance of conducting epithelia as a link between nervous and non-nervous structures. Electrophysiological studies of epithelial conduction begun by Mackie (1965), were first directed towards hydromedusae, as Spencer (1974) pointed out. Since then, a review by Anderson (1980) has incorporated data from sessile tunicate larvae, adult larvaceans and salps (as well as that from amphibian embryos and larvae). Lungfish embryos and young larvae (not surprisingly) also show propagated action potentials in the skin (Bone et al., 1989). No doubt the ciliated embryos of other fish groups (e.g. *Polypterus*) will prove to be similar, and skin cells capable of action potential propagation will be seen as a primitive vertebrate character.

It is certainly striking that epithelia are capable of propagating action potentials at velocities up to 50 cm s^{-1} (Table 1) and that in salps they can both elicit escape reactions in a suitable direction, and also can be driven by nerve fibres innervating epithelial cells (Anderson et al., 1979). In chains of salp blastozooids, escape reactions are transmitted along the chain by alternating neuro-epithelial and epithelio-neural synapses (Anderson & Bone, 1980). In this remarkable way, if the anterior individual of the chain is stimulated by touching a solid object, the entire chain reverses, whilst if the hindmost individual is touched, the whole chain accelerates forwards. Since there are several predators of salps, e.g. stromateoid fish and sea turtles, (see Harbison, 1998) strong stimulation at

Table 1. *Some examples of epithelial conduction in gelatinous plankton.*

Animal	Duration (ms)	Conduction velocity (cm s⁻¹)	Authority	Function
Dendrodoa tadpole larva	200–400	6–8	Mackie & Bone, 1976	Blocks swimming
Salpa outer epithelium	20	17	Mackie & Bone, 1977	Involved in escape reactions of individuals and of blastozooid chains
Oikopleura	40	40	Bone, 1985	Escape swimming evoked
Hippopodius	35–40	35–40	Bassot et al. (1978)	Blanching, crumpling, inhibits swimming
Chelophyes	35–50	35–50	Mackie & Carré, 1983	Escape swimming evoked

either end results in all its individuals separating so that the predator cannot simply eat its way gradually along the chain. Perhaps it is to avoid this possibility that the links between individuals in the chain have to be frangible as well as supporting communication along the chain.

The distribution of epithelia capable of propagating action potentials is of some interest, as are its possible functions. In the tunicates, for example, conducting epithelia are found in the inner and outer epithelium of salps (the function in the inner epithelium is not known, see Mackie & Bone, 1977); in the inner epithelium of the early old nurse stage of *Dolioletta gegenbauri* (where its function is likewise unknown); in oikopleurid larvaceans and in a single ascidian tadpole larva. Despite special searches, conducting epithelia have not been found in adult ascidia, in *Pyrosoma* at any stage, nor in any other of the complex stages in the doliolid life history.

In cnidaria, conducting epithelia are known only in hydrozoan jellyfish and siphonophores. They are absent in scyphozoans (where gap junctions linking cells are lacking). Anderson (1980) concluded that the production of epithelial action potentials may have arisen independently on several occasions, and Mackie (1970) has produced plausible suggestions for their development.

The initiation of epithelial action potentials has been little studied, and it is here that 'preparations' provided by gelatinous plankton may prove of interest to physiologists working on 'conventional' preparations. For instance, rather unexpectedly, despite being only 1–2 μm thick, larvacean skin cells have been shown to be suitable preparations for long term intracellular recording of skin impulses, (Bone, 1985). It is possible to record generator potentials summing to give rise to action potentials, and although only preliminary patch clamp records have been made, it appears from Na⁺-substitution experiments that these epithelial action potentials are carried by Na⁺ as they are in mammalian spindles. The accessibility of larvacean skin, as compared with the mammalian spindle is evident, and further studies are desirable to characterise the stretch receptor channels involved.

6. DIFFERENT KINDS OF MUSCLE FIBRES

The wide range of animals in the gelatinous plankton naturally offers a wide range of types of striated muscle fibre, a diversity greater than that found in land animals. Some of this range has proven useful for general physiology. There are also, in some ctenophores, interesting very large (unstriated) smooth muscle fibres. These 'giant' smooth muscle cells have been examined in the predatory ctenophore *Beroe* at the ultrastructural level and electrophysiologically (Hernandez-Nicaise et al., 1980; Bilbaut et al., 1988). Remarkably, as the latter authors show, muscular co-ordination during the rapid gulps by which *Beroe* ingests its prey depend entirely on interactions between muscle fibres with different channel properties. Two of these fibre types have different arrangements of the internal sarcoplasmic reticulum (SR) Ca^{2+} stores (Cario et al., 1995).

In all groups of the gelatinous plankton that have been examined, excitation-contraction coupling in striated and unstriated muscle fibres contrasts with that in vertebrates, for the muscle fibres require *external* Ca^{2+} to contract even if there are internal Ca^{2+} stores (Inoue et al., 1994; Lin et al., 2000) or sarcolemmal invaginations equivalent to the vertebrate transverse tubular (T) system, but even in such cases (as in appendicularians for example (Bone et al., 1977), external Ca^{2+} is required for contraction.

Cnidaria might be expected to show the simplest kind of striated muscle fibre, as found in medusae, though their structure has been studied in only a few

forms. In those where the locomotor musculature has been examined (*Polyorchis, Aglantha*) there appears to be a sarcoplasmic reticulum (SR) consisting of sub-sarcolemmal cisternae. In *Aglantha* there is a sparse SR with a few subsarcolemmal 60 nm vesicles (Singla, 1978), whilst in *Polyorchis* these are apparently Ca^{2+} stores involved in excitation-contraction coupling (Lin & Spencer, 2001). In the locomotor muscle sheet of diphyid siphonophores the situation is curious (Bone et al., 2000). Tubules within the muscle cells morphologically equivalent to an SR are absent, but there are regularly arranged tubules throughout the myofibril array that are invaginated from the bases of the muscle cells. They are thus morphologically equivalent to the tubules of a transverse tubular (T) system. Yet contraction is blocked by Co^{2+} provided both sides of the myoepithelium are accessed by Co^{2+}. Caffeine has no effect, thus these tubules do not seem to be equivalent to an internal Ca^{2+} store.

Even more curious are the tubular arrangements within the cross-striated locomotor muscle fibres of chaetognaths. Here the morphological equivalents of an SR and a T-system are present, and coupled with what appear very similar to vertebrate-like end feet composed of ryanodine receptors (Duvert & Salat, 1979, 1980). Yet physiological studies (Tsutsui et al., 2000) indicate that internal Ca^{2+}, stores are lacking. Savineau & Duvert (1986) have demonstrated cytochemically that the SR contains Ca^{2+}, so that it appears that some essential step in the excitation-coupling process seen in vertebrates is lacking and that the role of the SR may simply be just the sequestration and release of Ca^{2+} into the extracellular space of the T-system.

The complexity of these invertebrate muscle fibres from the gelatinous plankton rivals that of vertebrate fibres, but what seems to be the simplest possible striated muscle fibre is seen in the small tunicate *Doliolum*. *Doliolum* locomotor fibres are obliquely-striated, very rapidly contracting, and lack any kind of internal tubular system equivalent to an SR or T-system (Bone & Ryan, 1974). Muscle action potentials are carried by Ca^{2+} subsequently extruded from the fibre by a Na^+-Ca^{2+} exchange mechanism (Bone et al., 1997; Inoue et al., 2002). The *Doliolum* locomotor muscle fibre therefore provides an unambiguous example of muscle activation *solely* by external Ca^{2+} entry, which is not complicated by possible internal Ca^{2+} stores, as for example in vertebrate myocytes.

Since it is agreed that pelagic tunicates are derived from sessile ascidians (Swalla, 2001), it might be supposed that it was reasonable to construct a *morphological* series of complexity from the smooth muscle cells of adult ascidians (see Nevitt & Gilly, 1986), to the simple

doliolid obliquely-striated fibres and thence to the cross-striated salp fibres (Bone & Ryan, 1973) which have a conspicuous SR coupled to the sarcolemma but lack a T-system. The situation in appendicularia seems to depend on species size, (or rather muscle dimensions) for the larger oikopleurids have a T-system as well as an SR, whilst the much smaller fritillariids lack the T-system (Bone et al., 1977).

Romanes (1876) observed facilitation in the muscles of medusae, comparing it to that seen in frog muscle, but other means of changing muscle responses are seen in cnidaria. These range from the broadening of the presynaptic spike reducing junctional potential amplitude (Spencer et al., 1989) to increased duration of the muscle action potential as the hydrozoan medusa *Polyorchis* increases in size, which increases contraction duration since both Na^+ and Ca^{2+} are involved in the action potential (Spencer & Satterlie, 1981). Perhaps the most striking change in action potential amplitude and duration is seen in some diphyid siphonophores during short bursts of jet propulsion (Chain et al., 1981). In both, it is the duration of the action potential itself that determines Ca^{2+} entry, hence the response of the muscle. The initial action potentials of the burst are rapid events, but the slow rate of re-activation of the inward K^+ channel leads to successively larger and longer-lasting action potentials during which muscle contractions increase and so too the instantaneous velocity of the colony increases.

7. FEEDING

The feeding methods of gelatinous plankton have not had much impact on general physiology, with one exception. The planktonic cnidaria, ctenophora and chaetognatha are almost entirely carnivorous, feeding on copepods, larger arthropods, larval fish, and each other. Naturally, they themselves are prey for many organisms (Arai, 2005). The few non- or only partially carnivorous organisms are cnidaria relying in part for nutrition on symbiotic algae (e.g. *Velella* and *Cassiopea*), and those capable of taking up dissolved nutrients. Many are 'ambush' predators, floating motionless or moving very slowly awaiting their prey to blunder into dangling tentacles, but others make efforts to attract their prey with remarkable lures (Purcell, 1980), or undertake swimming behaviour to increase the area of water fished, as in the veronica swimming pattern of diphyid siphonophores (Mackie & Boag, 1963) and the swim and sink pattern of some chaetognaths (Feigenbaum & Maris, 1984).

Grazing filter-feeders in the gelatinous plankton have been less studied, but studies of the capabilities of the delicate mucous-net filters produced by pelagic

tunicates which enable them to capture small food particles (in some cases, sub-micron particles), have suggested a problem with the theories developed for fine-mesh net filters. The theory of operation of such filters (see Loudon, 1990) was based on values from the nets of hydropsychid caddis flies, and suggested that the fibres of the filter must be so 'sticky' that any particle touching them will be caught and ingested. However, as Loudon pointed out, agreement with theory could only be obtained by assuming that only 0.2% of the particles touching the filter were captured. Pelagic tunicates probably provide a better test system for the operation of fine filter nets than caddis flies, and further work is needed to resolve their operation (reviewed by Bone et al., 2003). At present, the construction of such 'mucous' filters from what seems to be a protein core coated with mucopolysaccharides, is incompletely understood (Bone et al., 2002). When it is, it may provide a possible technique for constructing cheap small mechanical filters.

The other group which uses mucus to capture particles are the pelagic thecosomes, some of which deploy very large outspread mucus sheets which they ingest when food particles have been collected (Gilmer, 1972). Nothing is known of the fine structure of these remarkable sheets of mucus.

8. TOXINS

Toxins from anthozoans have been used to dissect ion channels in the excitable cells of vertebrates, as have those from snakes, arachnids and scorpions. Palytoxin from a Hawaiian coral (*Palythoa*) has perhaps been the most widely used cnidarian toxin (see Oshida et al., 1983). So far, less use has been made of medusan toxins, though a glutamate blocker has been extracted from *Physalia* and neurotoxins from *Chrysaora* and *Aurelia* have been found to activate cation channels (Catterall, 2000). *Physalia* toxin stimulates Ca^{2+} influx into various cell types, probably by permeabilising the plasma membrane (Edwards et al., 2000).

No use has been made of toxins from other cnidaria such as siphonophores, where the recent discovery that the Na^+ channels of isolated myoepithelial cells are sensitive to tetrodotoxin (TTX) is unique in cnidaria (Inoue et al., 2005). Nor have the toxins been used from other groups such as chaetognaths, at least some of which utilize bacterial (TTX) to immobilize their prey (Thuesen et al., 1991).

Naturally, because of its dangerous effects on the human autonomic system, the effects of the venom of the cubomedusan *Chironex fleckeri* have been much studied, (see Burnett et al., 1998) though not yet used in experimental physiology, so far as I am aware.

9. PHYLOGENETIC CONSIDERATIONS AND EVO-DEVO

Kowalewsky's discovery of the ascidian tadpole larva (Kowalewsky, 1867), naturally disposed some influential zoologists to accept rather uncritically the ideas of recapitulation of phylogeny in ontogeny. At the beginning of the past century many still accepted them which is why Cherry-Garrard and his companions undertook their mid-winter trip to collect eggs of Emperor penguins (Cherry-Garrard, 1922). More recently, such ideas have been replaced by a less dogmatic approach, where molecular information rather than (and in addition to) morphology is used to seek genetic relationships and the evolution of structural and biochemical features. It is not too much to say that medusae and the ascidian tadpole larva have played an important role in the search for the origin of conserved developmental regulatory mechanisms such as the Hox gene cluster (Ferrier & Holland, 2001). Jellyfish have offered interesting perspectives on the possible evolution of the eye, mesoderm, neurons and striated muscle. Two small hydrozoan medusae *Podocoryne carnea* (without eyes) and *Cladonema radiata* (with eyes) have been used as models by Volker Schmid and his colleagues for investigation of different gene families, in particular, the homeobox genes involved in the conservation of upstream regulatory mechanisms in eye development (Stierwald et al. (2004)). Similarly, the cubozoan medusa *Tripedalia cystophora* has been used for eye control genes (Piatigorsky & Kozmik, 2004). *Tripedalia* and other cubozoans are exceptional in having image-forming camera-type eyes, perhaps to aid them to avoid obstacles in the neritic environment (Coates, 2003). Furthermore, molecular evidence from *Podocoryne* suggests that muscle and nerve cells are closely linked in evolution, perhaps derived from myoepithelial cells (Seipel et al., 2004).

As Spring et al. (2002) point out, pre-bilaterian organisms (present day Radiata) differ from the Bilateria because they have not devised mesoderm. Yet jellyfish possess muscle derived from a larval structure, the entocodon, a 'mesoderm-like' proliferative layer, and they also possess Twist genes (expressed mainly in the entocodon) involved in mesoderm formation in *Drosophila*. Castanon & Baylies (2002) discuss the changes in Twist function from its probable functions in jellyfish. Again, as Cole et al. (2004) show, the high molecular wt. serine proteinase inhibitor serpin 'jellypin' obtained from the jellyfish *Cyanea*, forms stable enzyme-inhibitor complexes with human serum proteinases, suggesting that the coevolution of serpin structure and inhibitory function predate the separation of Bilateria and Radiata.

Ascidian larvae have naturally been intensively studied, and following the almost complete decryption of the *Ciona* genome in 2002, with about half of the protein-coding genes found in humans, such features as the troponins of the caudal muscles (Cleto et al., 2003) have been examined to show the ancient origins of the chordate caudal musculature system.

Quite apart from an interest in the relationships of various 'lower' groups to each other, a spur to work on medusae and other gelatinous planktonic forms is the possibility of gleaning information about the function and evolution of important physiological aspects of 'higher' groups. For instance, the evolutionary modifications and structures of ion channels have attracted much work on medusae, as the starting point for voltage-gated channels in 'higher' forms. Thus following earlier work, Anderson & Greenberg (2001) have reviewed several types of voltage-gated channels, whilst Goldin (2002) has confined his review to the voltage-gated Na^+ channel. It is worth noting, that voltage-gated Na^+ channels are not confined to metazoan neurons, occuring in a single protozoan, (Febvre Chevalier et al., 1986) and in sponges (Leys et al., 1999). In addition to the light thrown on voltage-gated channels in higher forms, such studies are of considerable interest for it is via such data that we can gain some idea of the form and capabilities of the apparently diverse animals in the Precambrian over 550–600 mya (Chen et al., 2002). Here, the combined efforts of paleontologists and molecular biologists, as for example Erwin & Davidson (2002), have been of particular interest in compiling scenarios of animal life at the Vendian-Cambrian boundary, (see Miyata & Suga, 2001) for an interesting view of the periods of gene duplications.

10. CONCLUDING COMMENTS

There are likely several topics which have been omitted in this short review, and I apologise in advance to those physiologists and others whose work I have not recognized to have influenced general physiology. The choice of topics has been entirely my own, and no doubt has therefore been biased towards my own interests.

It is quite clear that Shimomura's work with his colleagues on aequorin and GFP has been by far the most significant contribution to date from the gelatinous plankton, probably followed (not too closely) by the results of work on the neuromuscular systems of medusae and pelagic tunicates. Perhaps the sensible conclusion to be drawn is that work on even the most unlikely and least-known groups of gelatinous plankton may unexpectedly yield dividends. Physiologists could well be divided into those who prefer to increase detailed understanding of the rich veins that have already been successfully worked for generations, and prospectors who seek outliers in the field. Both are valuable, and those engaged in the detailed study of a few 'model' organisms should not despise (or fail to give occasional grants to) the prospectors.

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