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CONDUCTION IN THE NERVE-FREE EPITHELIA OF SIPHONOPHORES

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SYNOPSIS. Optical and electron microscopy leave little doubt that nerve and muscle fibers are completely absent from large stretches of the epithelium covering the swimming bells in siphonophores (Class Hydrozoa). Behavioral experiments show that these regions must conduct, and electrophysiological evidence of propagated depolarizations in the epithelial cells has been obtained. Conduction velocities are in the order of 20-50 cm/sec, and a refractory period of 2-3 msec has been measured. Conduction is non-decremental and unpolarized.

Non-nervous conduction probably occurs in other siphonophore tissues (two examples are discussed), and it may be important in the behavior of many Hydrozoa.

The possibility that non-nervous conduction plays a part in the behavior of coelenterates has often been discussed (e.g. Hadzi, 1909; Parker, 1910; Pantin, 1956; Horridge, 1958; Mackie, 1960 a, b, 1964; Passano, 1963; Josephson, 1965). Workers have been forced to consider this possibility in cases where they could not find nerves in regions where the behavior suggested that conduction must occur, or where a single system of interconnected neurons seemed inadequate to account for the complexity of behavior. Although non-nervous conduction has remained hypothetical, many workers evidently regard it as a reasonable hypothesis, albeit one difficult to prove.

Comprehensive proof of non-nervous conduction ideally requires (a) behavioral evidence of conduction across the region in question, (b) demonstration of electrical events associated with the conduction, and (c) histological evidence that nerves are absent. Of these three types of evidence the last is usually hardest to provide, for unless the tissue is exceptionally simple histologically and is also easy to prepare for microscopical examination, it is difficult to be certain that failure to find nerves may not just mean that the nerves are there but have gone undetected. Many coelenterate tissues are complicated either by folding of the layers or by the presence within the epithelio-muscular sheet of a variety of cell types, particularly cnidoblasts and interstitial cells, which clutter up the picture. *Hydra* is an example of this. While the electron microscope certainly helps in the positive identification of nerve cells in such

complex tissues, when it comes to proving that nerves are absent, one is not much better off than with the light microscope.

Fortunately, however, there are certain tissues which are not only histologically uncomplicated, but are also easy to prepare in a form where their structural details are accurately shown, and where the worker can, in consequence, finally make an unqualified statement about the absence of nerve cells. The siphonophores offer a number of such cases, and in several of them the absence of nerves is as definite as any histological fact can be. In one of these tissues, the exumbrellar epithelium of the swimming bells, it has recently proved possible to obtain satisfactory evidence of conduction from physiological studies. As a result, we are now in a position to elevate non-nervous conduction to the status of a demonstrable phenomenon and also, since there are no muscle fibers in these cells, to characterize it as an epithelial type of non-nervous conduction in contrast to the myoid conduction that has been postulated to occur in epithelio-muscular cells (Hadzi, 1909). Before describing these results, I will mention two cases from my earlier work, in which non-nervous conduction is a serious possibility but where the evidence to prove it is incomplete.

THE FLOAT OF *Physalia*

The float consists of an outer case (codon) and an invaginated inner tube (saccus). Each of these structures consists of a layer of ectoderm and a layer of endoderm sepa-

rated by mesogloea. Muscle fibers are present in the epithelial cells in all four layers. The float is an active structure, capable of assuming a variety of postures involving both regional and general contraction. It would be reasonable to expect to find a nerve plexus associated with each layer. However, neurons have only been found in one layer, the codon ectoderm. Evidence for the absence of neurons from the other three layers can be put under two headings (Mackie, 1960b):

1. Silver impregnations were carried out with material from all four layers of the same animal together in the same batch. Nerves were shown only in the codon ectoderm, which is actually thicker and more folded than the other layers, and is thus potentially more difficult to stain by silver methods than are the other three layers.

2. The three other layers, particularly those of the saccus, make natural whole-mount preparations, and have been stained by a variety of methods. Visibility is so good that one can count and measure the chromosomes in mitotic nuclei (Fig. 1B) and detect chromosomal aberrations (Fig. 1C). In the best examples, it is possible to examine lengthy strips of tissue comprising hundreds of cells, and to relate each

nucleus to a particular cell territory in the nuclear layer of the epithelio-muscular sheet, as shown by the polygonal pattern of the cell membranes (Fig. 1A). Nerve cells if present would be conspicuous from the size and shape of their cell bodies and from their positions relative to the epithelio-muscular components, even if the neurites were not stained.

Although coelenterate nerves are reputed to be, and often are, difficult to demonstrate, there are certain places where they are very easy to see and can be shown without the use of special neurological techniques. If nerves existed in the saccus of *Physalia*, this would be a nearly ideal place in which to study their fine structure and interconnections. (An ideal material would be free of muscle fibers.) If it could be shown physiologically that these layers conduct, one would have an excellent, robust preparation in which to investigate non-nervous conduction.

THE SUBUMBRELLAR MUSCLE SHEET OF SWIMMING BELLS

The swimming bell is a modified medusoid member of a siphonophore colony. It lacks tentacles, gonads, marginal sense organs, manubrium, and the radial muscle which, in medusae, is associated with the manubrium. Radial (unstriated) muscle is, however, present in the margin where it serves to adjust the velum during swimming movements (Mackie, 1964) or to curl up the margin in a protective response in forms such as *Hippopodius* (Fig. 7).

The subumbrellar ectoderm consists of a single layer of cells of the epithelio-muscular type. The muscle fibers lie next to the mesogloea, run more or less circularly, and show periodical striations (Fig. 2B). In suitably fixed material, the whole layer can be removed as a sheet along with its mesogloea basement membrane, and can be mounted whole after staining. The underlying endodermal sheet can also be removed and examined. Chun's (1882) contention that there is no subumbrellar nerve plexus present can be confirmed on the following grounds:



FIG. 1. *Physalia* float tissue (Scale, 10 μ). A. Epithelio-muscular sheet showing nuclei (n), cell membranes (cm), and muscle fibers (mf); chrome-osmic fixation, iron haematoxylin. B. Prophase chromosomes. C. Anaphase, with outlying chromosome and fragment (arrow) not undergoing division. B and C are stained in thionin.

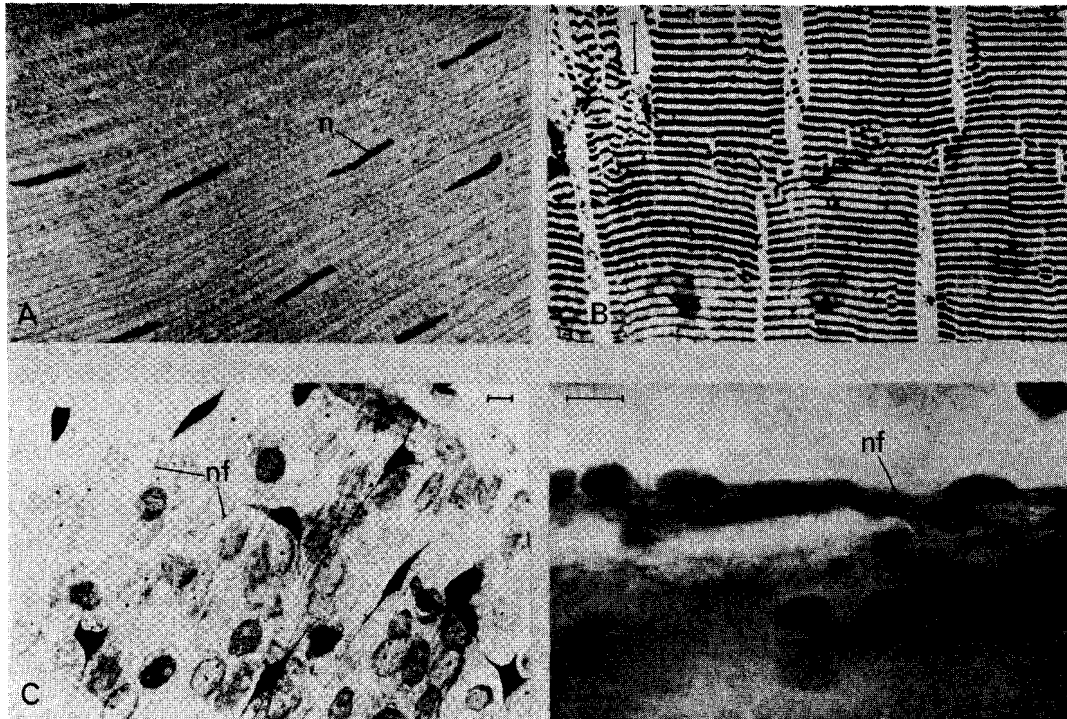


FIG. 2. Subumbrellar muscle sheets and associated nervous tissue (Scale, $10\ \mu$). A and B. Muscle sheets from the nectophores of *Forskalia* and *Chelophyes* respectively, Ehrlich's haematoxylin (A), and Holmes' silver stain (B). C. Subumbrellar muscle

sheet and nerve plexus in the free, medusoid gonophore of *Hippopodius* (iron haematoxylin). D. Marginal nerve ring of *Hippopodius* (Holmes' silver). n, nucleus; nf, nerve fibers.

1) The nuclei are all of one shape and size, are distributed in roughly geometrical patterns, and react the same to stains (Fig. 2A). They quite clearly belong to a single class of tissue, the epithelio-muscular cells.

2) Nerve fibers are not shown in the region by silver methods, although in the same preparations the nerve fibers comprising the marginal nerve rings are selectively stained (Fig. 2D).

3) The subumbrellar nerve plexus is relatively easy to see in the smaller hydromedusae and in medusoid gonophores by phase contrast and in preparations stained in various ways (e.g. iron-haematoxylin following chrome-osmium fixation (Fig. 2C)). These methods used on the swimming bell muscle sheet show no such evidence of nerve fibers or cell bodies.

I have suggested elsewhere (Mackie, 1960a) that the absence of a nerve plexus from this tissue is an indication that the

epithelio-muscular cells probably conduct the electrical impulses for their own contraction; the presence of a subumbrellar nerve plexus in hydromedusae is presumably correlated with the presence of radial muscle in the manubrium and the need for coordination between the manubrium and margin during feeding activities. It has not been definitely shown, however, that a propagated depolarization passes across the epithelio-muscular sheet prior to a contraction, although this seems far more likely than any other explanation for the rapid spreading of the response.

THE EXUMBRELLAR EPITHELIUM OF NECTOPHORES

Histology

The outer surface of the nectophore is covered from the muscular margin to the point where the stem is attached with a

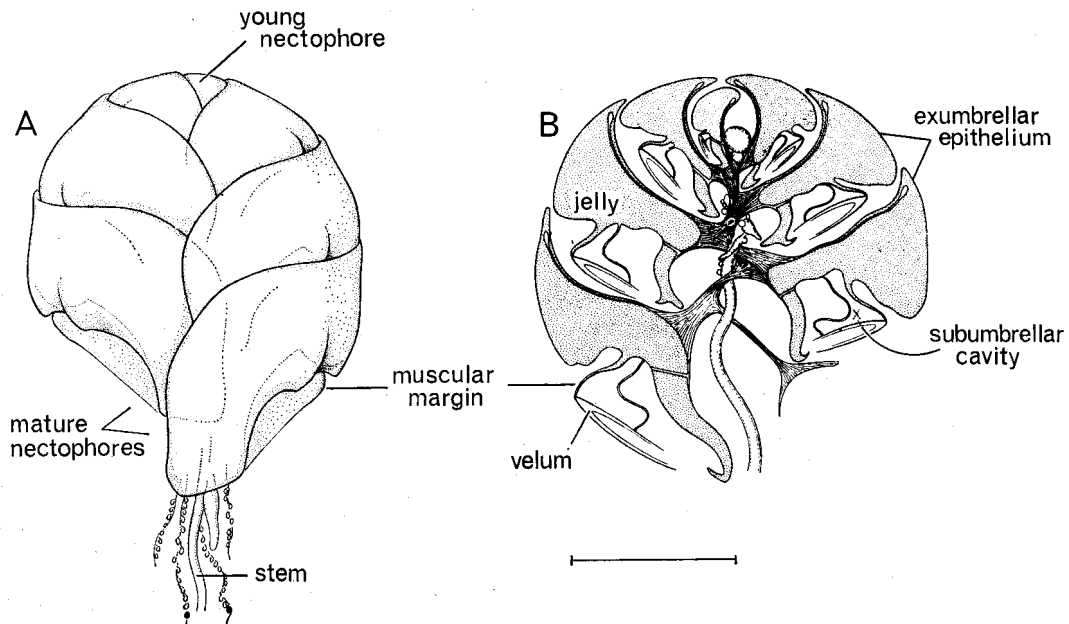


FIG. 3. *Hippopodius* (Scale, 1 cm). A. Intact, from life; B. Dissection, after Chun, 1897, Verh. deutsch. zool. Ges. 7.

thin, non-muscular layer of ectoderm cells, the exumbrellar epithelium (Fig. 3).

In certain Physonectae (e.g. *Nanomia cara* (Agassiz), *Forskalia edwardsii* (Köl liker)) the exumbrellar epithelium is infiltrated by nervous tissue either in the form of an open plexus or of a compact bundle or tract. Such a nerve tract is shown in Figure 4C. In the low power, phase-contrast picture (Fig. 4D), the nerve cell nuclei are shown as brightly shining objects spread out at intervals along the tract. For each neuron, there is about 1000 μ of nerve fiber at a cautious estimate; since these are bipolar neurons, a typical nerve process would be about 500 μ long. The tract lies beneath the ectodermal epithelium, and from the shape of the ectodermal nuclei in its vicinity it looks as if the whole tract may be enclosed within an epidermal sheath, although this requires confirmation by electron microscopy. In the surrounding regions, the exumbrellar epithelium consists of thin, flat, polygonal cells. Considerable detail can be made out by phase contrast, including the spiky projections from the cell membranes (Fig. 4A), and the basal apparatus of the ciliary comblets,

which consists of an arc of basal granules each with a bipolar rootlet, the whole apparatus being flattened in the plane of the epithelium and resembling a bird's foot (Iwanzoff, 1928). (The cilia beat in the direction of the "heel.") Details of this sort are usually beyond the range of the light microscope. It is reasonable to claim that if there were nerve cells or outlying nerve fibers running in the epithelium, they would be easily detected, and, indeed, in the limited regions where nerve cells do occur, they are easily seen; but large areas of the tissue show no nerve elements. Experimental studies on *Nanomia* showed that all parts of the exumbrellar epithelium were electrically excitable and could transmit excitation resulting in muscle action (Mackie, 1964). Excitation transmitted via the nerve tract was also demonstrated and shown to result in a different form of behavior.

Study of these physonectid genera is rather difficult because the nectophores are small, soft, and fragile, crumpling badly when fixed. Much more robust material is available in the calycophoran genera studied at Villefranche (*Hippopodius hip-*

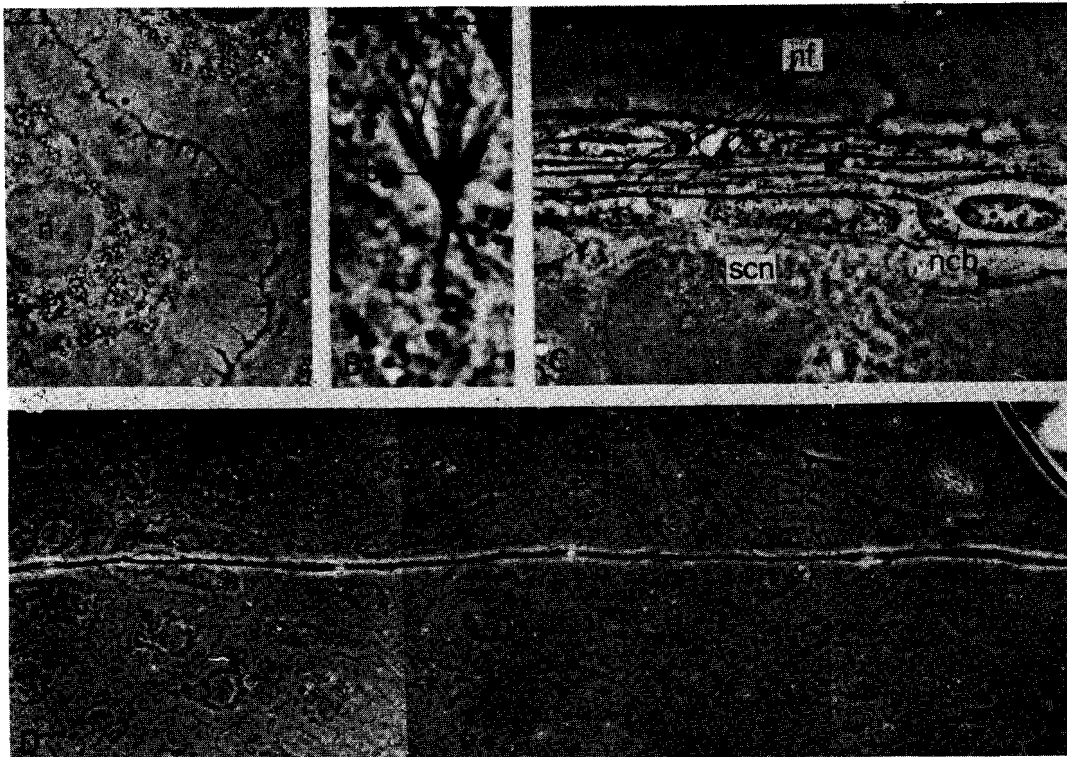


FIG. 4. *Forskalia*. Phase contrast pictures of exumbrellar epithelial whole mounts, osmic fixation, (Scale, 10 μ). A. Epithelial cell showing spiky projections from cell membrane (cm) into cytoplasm, granular material around the nucleus (n). B. Bird's foot pattern of basal apparatus of ciliary

comblet. Each basal granule (bg) has a rootlet (r) running in the plane of flattening of the epithelium. C. Nerve cell body (ncb) and nerve fibers (nf) running under epidermis. Nuclei of sheath cells (scn) curve around the fiber tract. D. A long stretch (0.9 mm) of the exumbrellar nerve tract.

popus (Forsk.), *Abylopsis tetragona* (Otto), and *Chelophyes appendiculata* (Eschscholtz)).

A thorough histological study was carried out on the exumbrellar epithelium of *Hippopodius*. Large areas of this tissue can be removed intact and mounted whole, making ideal preparations for study of minute cytological details. The tissue differs from that of the physonectids in lacking nerve elements completely, in being partially syncytial, and in having a greater abundance of secretory granules in the cytoplasm. There are no cnidoblasts, pigment cells, or interstitial cells to complicate the picture. There is only one cell type present, and it is free of muscle fibers.

Figures 5A, B, and E show syncytial and cellular regions of the epithelium. In the cellular parts mono-, bi-, and multinucleate

cells occur. The cellular region is located on the axial side of the nectophore around the point of attachment to the stem. Immediately around the stem attachment, the cells are relatively small, and their membranes are deeply inter-folded (Fig. 5D). Further out, the cells are larger and the membranes relatively straight (Fig. 5B). A sharp line of demarcation separates cellular and syncytial regions (Fig. 5A). The tissue is postmitotic from an early stage in the development of the nectophore bud. "Amiotic" stages such as those shown in Fig. 5E are probably sister nuclei still connected by a fusome (spindle relic). Ciliary comblets are present on the axial side of the nectophore. The arrangement of the basal apparatus resembles that found in *Forskalia* (Fig. 4B), the basal granules lying in arcs or in almost complete circles in a few cases.

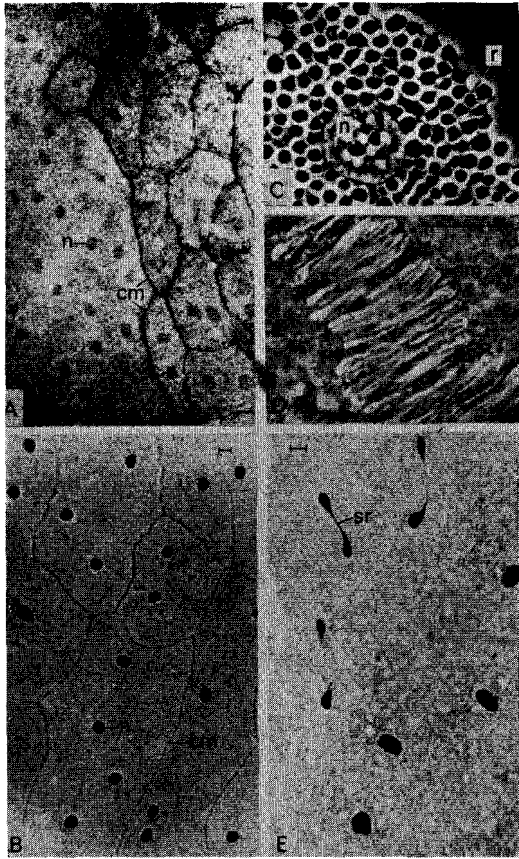


FIG. 5. Exumbrellar epithelium of *Hippopodius*. Surface views of whole tissue sheets. Fixation: osmic-acetic after acrolein, except E, which is Bouin (Scale, 10 μ). A. Boundary zone between syncytial region and cellular region, dark ground illumination. B and E. Phase contrast views of the two regions. C. Heavily granulated region, showing reticular appearance of cytoplasm, phase contrast. D. Region near stem attachment point, showing interfolded cell membranes, phase contrast. cm, cell membrane; n, nucleus; r, reticulum; sr, presumed spindle relic uniting two nuclei.

The cilia usually beat in synchrony within a combet, but different combets are not synchronized and they may beat at widely different frequencies (2-8 beats/sec) in different regions. The cytoplasm shows a variably granular nature. In the cellular regions the granules are small and relatively far apart, but in the syncytial regions they are larger, up to 3 μ in some cases, and are so abundant that they dominate the picture completely, even overlapping in some places. Iwanzoff (1928) found con-

siderable variation in the amount of granulation in the siphonophores he studied. The appearance of dense granules surrounded by a clear space in Figure 5C is a fixation artifact. In life, the material of the granules fills the spaces within the "reticulum" (non-granular cytoplasm).

Preparations of detached epithelium have been compared with numerous preparations made by removing the whole outer layer of the mesogloea along with the epithelium. Visibility is almost as good in such preparations as in those of the epithelium by itself. It is clear that there are no cellular elements such as nerves which might lie under the epithelium and remain stuck to the mesogloea when the epithelium is detached. The possibility that neurons run in between the cells in the cellular regions, following their contours exactly, can be eliminated, for intensive study has shown that there are no nuclei in these intercellular regions at any stage in the development of the nectophore. In the syncytial parts, the possibility does not arise. In brief, we have here a tissue consisting of one cell type, one cell thick, showing a diagrammatic simplicity of structure, easy to prepare and examine in all regions, and lending itself to observations at the limit of optical resolution. In the same tissue of related siphonophores nerves do occur, and are easy to see. Their absence from this tissue is all the more striking.

In *Chelophyes* and *Abylopsis*, the exumbrellar tissue has been examined rather less exhaustively than in *Hippopodius*, but sufficiently to show a general kinship of structure. Parts of it are syncytial, granulations are present, and ciliary combets are found. The granulation in *Chelophyes* is rather patchy (Fig. 6A). Electron microscopy of osmium fixed material embedded in Epon and stained with uranyl acetate shows the granules as masses of amorphous material contained within membranous sacs. The membranes show the usual unit membrane structure. Presumably, in pictures such as Figure 5C, the reticulum represents adherent sac membranes for the most part. Mitochondria (Fig. 6C), Golgi element (Fig. 6D), and various small membrane-

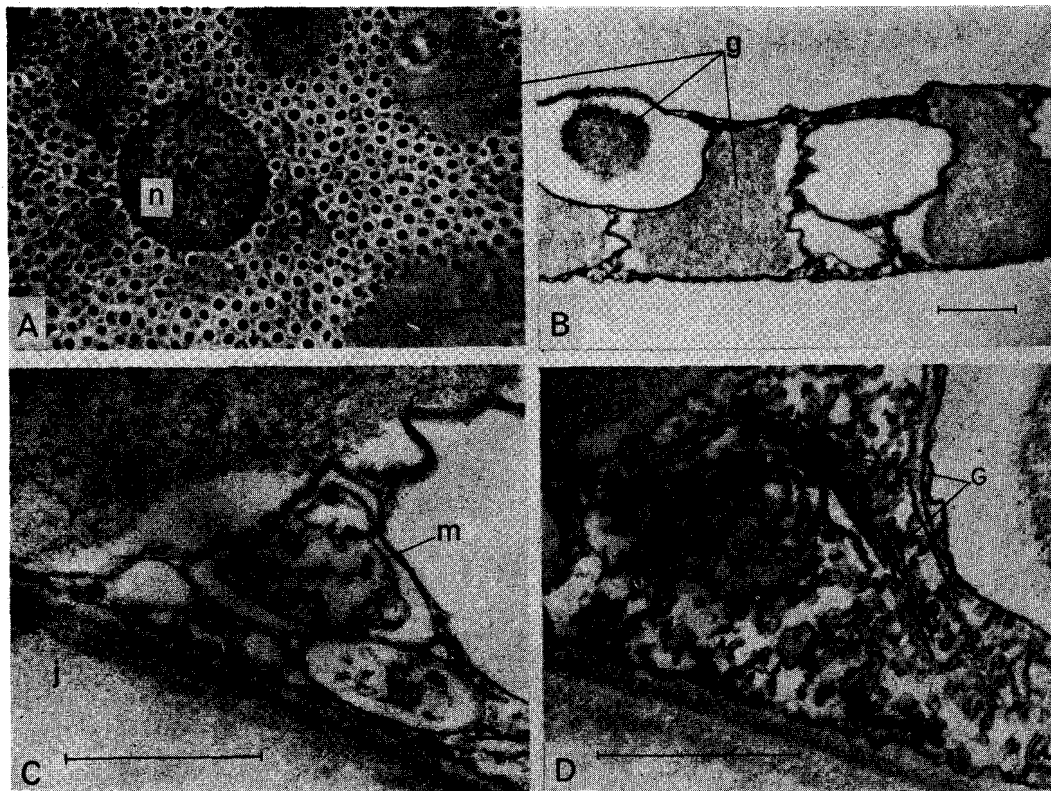


FIG. 6. Exumbrellar epithelium of *Chelophyes*. A. (Scale, 10 μ .) Phase contrast view of osmium-fixed strip preparation showing a nucleus (n) and surrounding areas of granular and non-granular cytoplasm. B, C and D. (Scale, 1 μ .) Electron micrographs of osmium-fixed epithelium showing the granules (g) as amorphous masses enclosed within

membranous sacs. Mitochondria (m) are interspersed among the granules, and Golgi membranes and vesicles (G) have been found in the vicinity of small accumulations of granular material. The epithelium in C and D is still attached to the gelatinous mesogloea ("jelly"-j).

lined sacs, vesicles, and tubules presumed to represent endoplasmic reticulum, are found interspersed among the large granules, particularly on the mesogloal side. There is some indication that the granules arise in association with the Golgi apparatus. The cell membrane of the syncytium on both sides appears unspecialized, although on the free side, here and there, villous protuberances have been seen.

The peculiar appearance of this tissue is due to the unfamiliarity of an arrangement wherein a cell containing secretory granules up to 4 μ thick is spread out flat until its thickness is limited by that of the granules. The granules are forced to jostle for elbow room within the confines of a single layer. If the cell were drawn up into

a columnar shape it would look much more credible. The granules would probably be congregated at the free end. Since the granules are small and sparse in some regions, and absent in others, there is no reason for supposing that they have anything to do with conduction; our present evidence suggests an equal capacity for conduction in all regions of the epithelium.

Physiology

In an isolated nectophore of *Hippopodius*, when the exumbrella is touched or electrically stimulated without physical contact with the electrode, a response is seen in the muscular margin, consisting of the contraction of radially arranged muscle fibers and consequent rolling inward of the

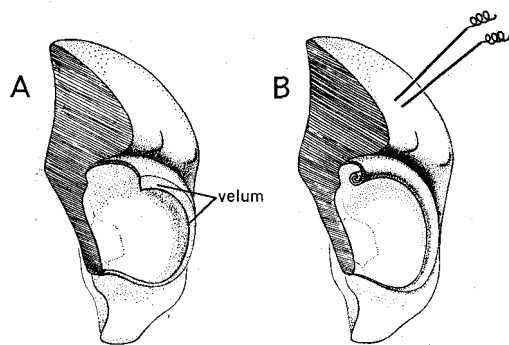


FIG. 7. Half-nectophores of *Hippopodius*. A. The margin and velum are relaxed and extended. B. Following an electrical shock delivered on the exumbrella, the margin rolls inward.

free edge of the margin and velum (Fig. 7B). Even if the radial muscle is already somewhat contracted, a twitch can usually be observed in it following stimulation. The electrical potentials accompanying this twitch can be recorded by an electrode placed on or near the margin. In fresh specimens, the delivery of a stimulus is followed after one or two seconds by the development of cloudiness over all regions of the exumbrella. This cloudiness transforms the nectophore from a transparent to an opaque object. It was previously speculated (Mackie, 1964) that this "blanching" effect and the muscle response at the margin were both the result of the passage of excitation across the exumbrellar epithelium. This now appears to be reasonably certain, although it has been found that the opacity takes place not in the epithelium itself but just underneath it in the surface layers of the mesogloea. Another phenomenon apparently associated with exumbrellar transmission in this form is a flash of luminescence, observed when the exumbrella is stimulated in the dark. Details of the blanching and luminescent responses will not be included here, since they appear to be secondary phenomena and are found only in *Hippopodius*. It may be said, however, that the development of cloudiness in the mesogloea, which involves the sudden formation of light-scattering granules, occurs irreversibly and locally when the epidermis is removed, as well as reversibly and generally following stimula-

tion. The granules can be eliminated by treatment with Versene (disodium ethylenediamine tetraacetate), suggesting the involvement of Ca^{++} . This is interesting in view of current theories of transmission in nerve and muscle, in which Ca^{++} influx during depolarization is held to occur and, in the case of muscle, may result in activation of the response system.

In nectophores of *Chelophyes* and *Abylopsis* (Figs. 8, 9), stimulation of the exumbrella leads to a marginal muscle response involving the velum and adjacent areas, sometimes spreading to the swimming musculature. In intact colonies of these two forms, the stem also contracts. Suction electrodes can be attached to the marginal muscle of the nectophores or to the stem. Potentials recorded at both sites were used as indicators of exumbrellar transmission, and the signals picked up were correlated with visual observation of muscle responses.

In all three forms, investigations were carried out to determine conduction velocity over the exumbrella (Fig. 8). Data for *Hippopodius* suggest a value of approximately 30 cm/sec at 21°C. Accurate estimation was made difficult by the fact that with the recording paper running at full speed (82 mm/sec), the distance between stimulus and response signals was still not more than a few millimeters, owing to the rapidity of conduction and the small size of the preparation. Further, it was not always easy to recognize the separate components of the muscle response, which is important for comparative purposes. Better records were made, using an oscilloscope camera, in experiments on *Abylopsis* and *Chelophyes*. Values of approximately 24 and 35 cm/sec respectively were obtained at 22°C. For a given position of stimulating and recording electrodes, response time remains constant, although the form of the muscle response varies. All these values were obtained by comparison of response times in tests where the shocks were given at a series of points at known distances apart, chosen along a line representing the shortest route across the exumbrella to the stem. A single shock is all that is required to send a propagated impulse over the

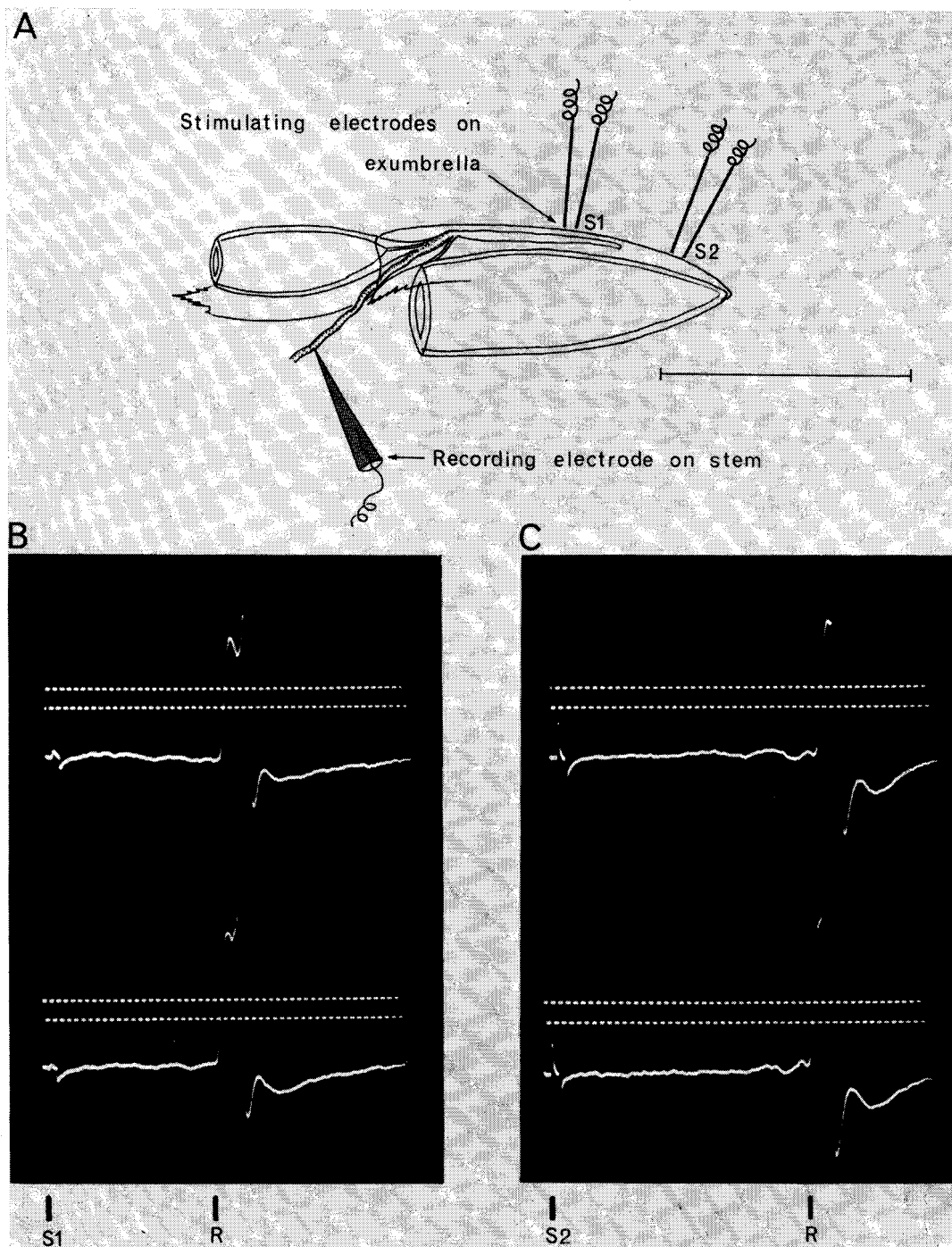


FIG. 8. *Chelophyes*. Method of measuring conduction velocity over the exumbrella. The time intervals represented by distances S1-R, S2-R (B and C) represent conduction time from points S1 and S2 on the exumbrella to the stem muscle whose response (R) is recorded with a suction electrode.

The difference between the two gives conduction time in the strip of exumbrellar epithelium between the two stimulating points. Time signals in milliseconds are included in the records. (The drawing of *Chelophyes* is modified from one by Fewkes, 1880.) (Scale, 1 cm.)

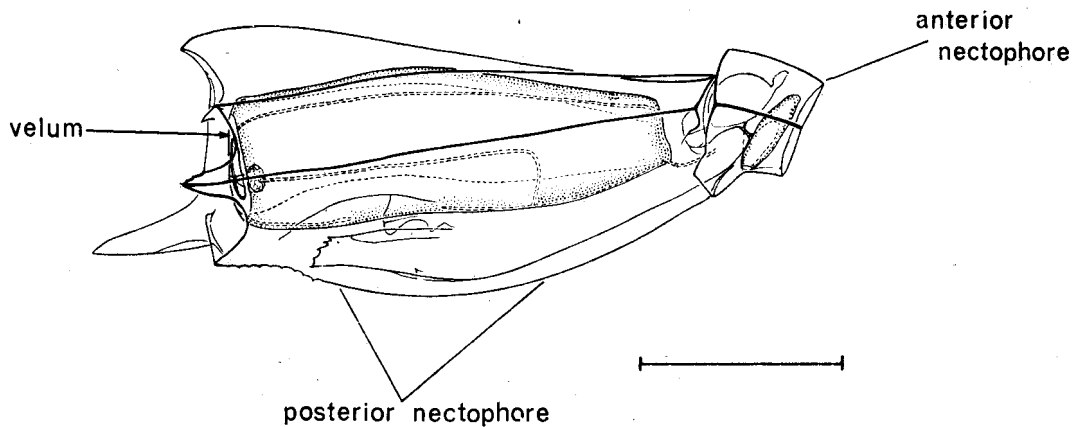


FIG. 9. *Abylopsis* (Scale, 1 cm). The large posterior nectophore was used for the experiments. The stem is not shown. (From Totton, 1954, slightly modified in the velar region.)

longest exumbrellar distances obtainable in these preparations (over 2 cm in *Abylopsis*, Fig. 9). In *Hippopodius*, cellular and syncytial regions appear to conduct in the same way, and no evidence for polarized or decremental conduction has been obtained either in this or the other two species.

For conduction to occur over the exumbrella, the covering epithelium must be in good condition. Shocks applied to regions where the epithelium was damaged or missing did not elicit responses. The epithelial sheet can be lifted off by repeated light stroking with a fine glass needle with a rounded tip. This can be done without visibly damaging the mesogloea surface. Under good conditions of lighting, using dark-ground or oblique illumination, the operation can be done critically and denuded areas can be prepared according to the investigator's whim. Sinuous conduction pathways can be created, and islands of intact tissue can be isolated by circumbrasion.

A number of experiments with *Hippopodius*, which lends itself particularly well to this sort of treatment, show that propagation must be at the surface of the exumbrella, and cannot be by any deep path through the mesogloea (Fig. 10). As soon as the epithelium is damaged or removed, conduction ceases. These observations could be explained in three ways: (1) the epithelium is the conducting material; (2) the mesogloea surface is the conducting

material, but it is damaged when the epithelium is removed and ceases to conduct; (3) conduction is a collaborative process involving both. We are not in a position to settle this matter at the moment, but for the purposes of discussion, we assume that the epithelium alone conducts. This

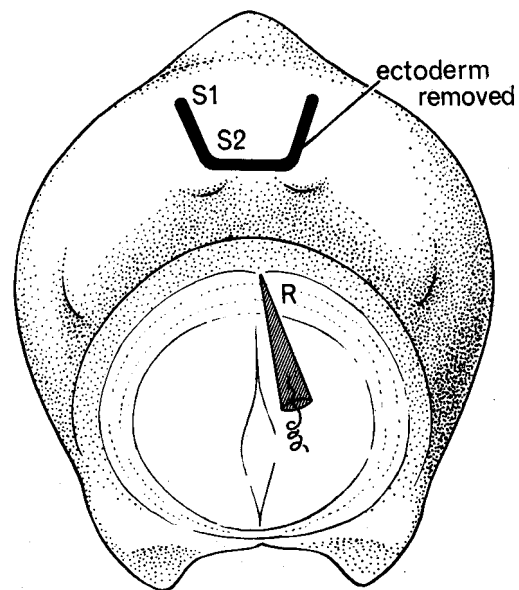


FIG. 10. Isolated nectophore of *Hippopodius*. The conduction time between stimulating point S1 on the exumbrella and recording point R on the margin is shorter than between point S2 and R, and, in general, is proportional to the length of intact exumbrellar epithelium between the S and R points. Experiments such as this indicate that there are no deep conduction routes through the mesogloea.

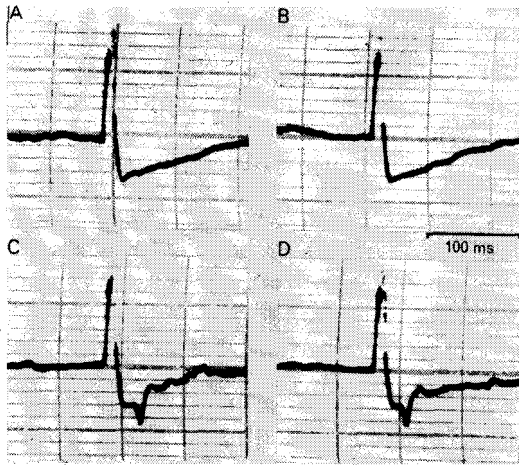


FIG. 11. Exumbrellar potentials recorded from *Hippopodius*. A and B are controls showing the shape of the stimulus artifact where the conducting tissue does not respond. C and D show a small potential following the stimulus which represents the passage of a propagated wave of excitation under the recording electrode.

seems far more likely than the other possibilities, in view of all that is known about conduction in electrically excitable systems, in particular its dependence on polarizable cell membranes. The mesogloea is a gelatinous extracellular product in these animals. It contains no free cells and is not penetrated by cellular processes. It contains collagen and what appear to be elastic fibers. It serves as a skeleton, giving the animal its shape and opposing muscle action in some cases. It also contributes to buoyancy, being lighter than sea water.

Direct recordings of exumbrellar potentials were successfully achieved using glass micro-electrodes. In these experiments, the muscular responding regions were either removed completely or were isolated from the exumbrellar epithelium by incision or by abrasion. Thus the only indications of conduction were the signals picked up with the electrode inserted in the epithelium. These signals are much smaller than those obtained from the muscular regions, but they were picked up fairly consistently in all three forms, and recorded either on paper (Fig. 11) or were photographed from the oscilloscope (Fig. 12). The time interval between stimulus and response signals varies directly with the distance between

stimulating and recording electrodes, and gives a direct value for conduction velocity. The highest reliable values obtained for *Hippopodius* were in the range 35-40 cm/sec. Some lower values in the region of 25 cm/sec may represent errors in measurement of distances on the paper records, where the response signal was not well defined or where it merged in with the stimulus artifact. On the other hand, it is worth considering that conduction may not always go by what (to the observer) appears to be the most direct route.

Direct readings on *Abylopsis* gave a figure of 24 cm/sec, confirming the values obtained indirectly using the muscle response as an indicator. Experiments on *Chelophyes* were hampered by calibration problems, but the conduction velocity can probably be higher than the figure of 35 cm/sec obtained earlier, and may be as high as 50 cm/sec. In all cases where a series of stimulus-response signals were picked up in one stimulating-recording

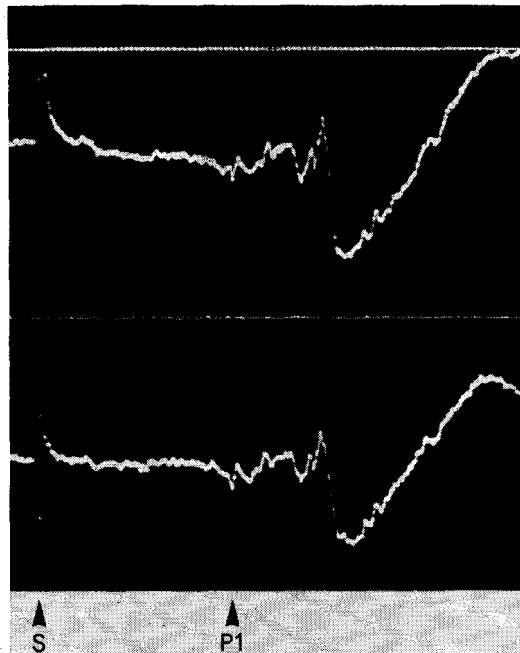


FIG. 12. Exumbrellar potentials recorded from *Chelophyes*. The stimulus (S) evokes a series of potentials of which P1 is the first and most constant in its time relation to the shock and in its shape. P1 is estimated at 30-50 μ V. (Time scale, above, in milliseconds.)

position, the time interval (i.e. velocity) was virtually constant. Inconsistencies in the values obtained in different recording positions appear to stem from inaccuracy in estimating distances over the exumbrellar surface, rather than from any other factor. The exumbrellar surface in *Chelophyes* shows deep ridges and furrows. There is also the possibility that unnoticed damaged patches existed in the area between the stimulating and recording electrodes, making the actual conduction route longer than the one estimated to be the most direct.

The form of the response as seen on the oscilloscope may be very simple, consisting of a single small potential such as P1 in Fig. 12. This small potential is a constant feature, occurring either alone or followed by other potentials. Conduction velocities were estimated using the P1 potential as a marker. The significance of the subsequent potentials is hard to explain. In many records they were not shown at all. However, where they did occur, they showed fair consistency in their time-relations and shape. They might be artifacts, but it is hard to imagine what could cause such an artifact.

Occasionally, signals resembling P1 were picked up when there had been no electrical stimulus. These were probably set off by slight accidental movements in the recording bath; the epithelium is essentially touch-sensitive, although for experimental purposes electrical excitation was nearly always used. There was no convincing sign of endogenous or pacemaker activity in the system.

When the stimulating electrodes are well placed, the recording electrode can be removed and reinserted in new positions repeatedly, and the signals can be picked up in nearly all of them. This suggests that if the conducting elements were neurons, they would have to be in the form of a very dense plexus, because the tip of the recording electrode is only $1.5\ \mu$ in diameter. Where signals are not picked up, the trouble usually lies in the positioning of the stimulating electrodes. If they are too far from the surface, a higher voltage shock

may be called for; if they are too near, their tips may damage the epithelium, calling for repositioning. The preparation is unusual in that it is often harder to stimulate it than to record from it.

Measurements of the refractory period of the tissue were made using direct recordings from the exumbrellar epithelium. The absolute refractory period lies between 2 and 3 msec. The refractory period of the exumbrellar-epithelium/stem preparation appears to be of the same order (Fig. 13).

DISCUSSION

All the evidence presented in this paper points to the exumbrellar epithelium as a non-nervous conducting tissue, functioning with high efficiency in the sense that it shows an all-or-none character, and conducts at a velocity and with a refractory period comparable to unmyelinated nerve fibers in vertebrates. It can conduct in any direction, and the excitation can apparently pass across cellular regions as well as syncytial in a through-conducting manner. In this respect it is reminiscent of heart muscle in a vertebrate, which is a multicellular, non-nervous tissue conducting and responding as if it were a single cell. The exumbrellar epithelium could be likened to a single giant axon spread out over the whole exumbrellar surface. Like the giant axon, it provides a motor route for the rapid effector responses involved in escape or protection (stem contraction, swimming, protective closure). In life it appears to be excited whenever it makes contact with an outside object which results in a mechanical impact of a sufficient order. It does not seem to have any capacity for endogenous activity.

One may ask—is this a special case of conduction in ciliated fields? It is still uncertain what part propagated electrical events play in the transmission of waves of ciliary activity, but there is some reason to suppose that certain ciliated tissues may conduct electrically, and without the involvement of nerves. Thus Satir and Miller (1961) report what appear to be a resting potential and rhythmic signals associated with ciliary beating from intracellular re-

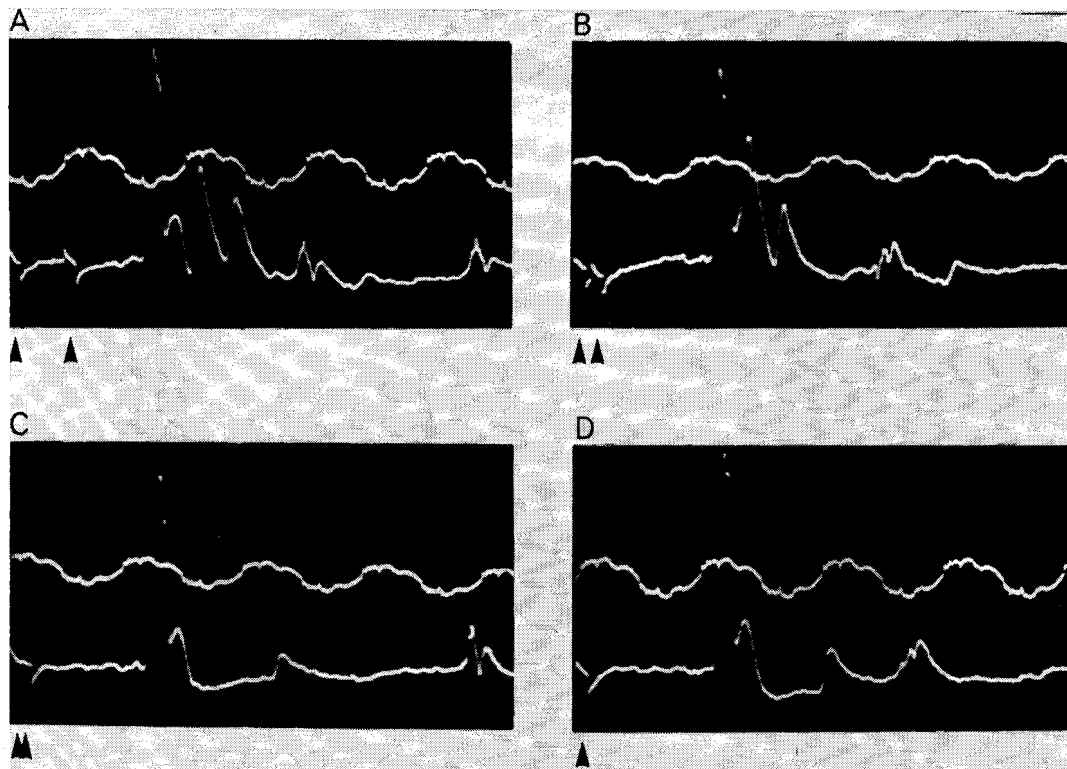


FIG. 13. Refractory period estimations, *Chelophyes*. The arrowheads mark shocks delivered at a point on the exumbrella. The following potentials represent the muscle response recorded from the stem. Intervals between shocks are 10 ms (A), 3 ms (B)

and 2 ms (C). D is a control, showing the response to a single shock, which is the same as the response to two shocks 2 ms apart (C), except for the small after-potentials which are not significant. A 50 cycle/sec signal is included in each record.

cordings from the gills of the clam *Unio*. Whatever the relationships of the conducting system in our present material, it is clear that it has evolved far beyond the needs of coordinating ciliary action, if indeed this ever was its function. Cilia are absent from extensive regions of the conducting tissue and, where present, are usually far apart and, apparently, uncoordinated outside the limits of individual comblets.

The siphonophores are a notoriously complicated and peculiar group in some ways, and the relevance of these findings to more "normal" material may seem remote. However, the complexity of the siphonophore is mostly on the level of gross morphology. The specialization of the individual members is often associated with histological simplification, rather than the reverse. Thus the conversion of a multi-

purpose medusa to a one-purpose nectophore has led to wholesale loss of components. The muscle sheets, nerve rings, and epithelia which remain differ from "normal" coelenterate tissue chiefly in being much less cluttered up with other cell types. They offer attractive material for the pure-minded histologist who wishes to study one thing at a time. Similarly, division of labor among the members of the colony gives the physiologist a chance to study certain basic functions in relative isolation.

It seems increasingly likely, in view of the results given here, that epithelial and epithelio-myoid conduction mechanisms are important in the Hydrozoa, supplementing nervous conduction in various ways. One comes to look upon nerves as serving a rather limited and specific role in the behavior, instead of being responsible for all

behavior. Their primary value would seem to be that they can provide insulated pathways for effector actions, in which the action has to be limited to a certain region or to a certain component of a generalized effector system which may at other times (as when excited non-nervously) act altogether as a unit. Added to their role as insulated transmission pathways, nerves may have specific roles as initiators of activity, acting as pacemakers (Passano, 1963).

It also seems likely to the author that the large potentials picked up in the stem following exumbrellar stimulation represent the continued passage of a wave of non-nervous excitation, this time in the membranes of the epithelio-muscular cells. The non-contractile epithelial cells of the exumbrella merge into the contractile epithelio-muscular cells of the stem through a transitional zone in which the muscle fibers are rudimentarily developed. One can suppose that the propagated electrical event goes right on across this zone into the stem cells, and that the large signals obtained there represent the normal membrane depolarization of a fraction of a millivolt, augmented by potentials from activated muscle fibers. There is a resemblance between these big signals (which may come spontaneously in bursts) and the pacemaker potentials from *Hydra* and *Tubularia*; all of them could be epitheliomyoid signals. In the septate desmosomes of *Hydra*, Wood (1961) found some indication that the outer, dense component of the unit membranes of the epithelio-muscular cells was continuous between one cell and the next. This may mean that, if these cells do conduct, excitation may pass from one to another electrotonically; Bennett (1964) has associated such fusion with a capacity for electrotonic spread in a number of tissues. Septate desmosomes occur between the cells in the salivary gland of *Drosophila* (Wiener, Spiro and Loewenstein, 1964), where they appear to be functionally associated with the low electrical resistance and high permeability known to exist across the junctions. Such a chain of cells connected by low-resistance junctions could serve as an evolutionary starting

point for the development of various types of non-nervous conducting tissues.

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