

Neurociliary synapses in Pleurobrachia (Ctenophora)

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With 7 plates (figs. 2 to 8)

Summary

Synapses of a typical form, with synaptic vesicles of 30 to 45 m μ diameter, on one side of a synaptic cleft of 10 to 12 m μ , are found near the bases of the long ciliated cells which make up the comb plates. The side containing the vesicles is interpreted as a section of a nerve fibre terminal in contact with the ciliated cell. Occasionally nerve-cell bodies, identified by similar vesicles, are encountered. These nerves are probably ramifications of the ectodermal nerve net which spreads over the whole animal and which transmits the inhibition of the ciliary waves when the animal is touched. Distinctly different nerve fibres, which run from the apical organ along the ciliated grooves to the end of the comb rows, are able to initiate a ciliary wave along the comb row. The excitatory nerves appear as rather empty axons which mingle with the bases of the ciliated cells. The question of the propagation of excitation between these axons, between the ciliated cells of a comb plate, and between comb plates, remains open, although regions where excitatory axons and ciliated cells make contacts between themselves and with each other have some resemblance to electrically transmitting synapses.

Introduction

THE comb plates of ctenophores have long been recognized as outstanding examples of the control of cilia by nerves. Following the anatomical account of Eimer (1873) there was a series of histological and structural studies, notably by Chun (1880), Hertwig (1880), Schneider (1892), and Samassa (1892) which laid the foundations for a series of physiological investigations of the control of the cilia. Although aware of the existence of nerve-fibres in proximity to the ciliated cells, and of the inhibitory response described below, Parker (1905) introduced his term 'neuroid transmission' to explain the propagation between the ciliated cells. By this term Parker meant the propagation directly from cell to cell, and in ctenophores he considered this a preferable alternative to transmission based upon mechanical contact of each cilium upon its neighbour, as proposed by Verworn (1891). Although first put forward on inadequate grounds by Eimer (1873, p. 46), the idea that the cilia are controlled by nerves has gained ground during this century, though still without proof. A temporary complete cessation of the beat follows a mechanical stimulus to the oral end in *Beroë* (Chun, 1880, footnote to p. 172; Bauer, 1910; Kinoshita, 1910; Gothlin, 1920, 1929). The response is spread from a stimulated point in any direction over the whole animal as if propagated by

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the ectodermal nerve net. This inhibition has been confirmed in *Pleurobrachia* and *Beroë* and at St. Andrews has been analysed by electrical stimulation of both species. A directionally homogeneous nerve net propagates the spread of the inhibition but physiological investigations leave the actual relations with the ciliated cells obscure.

Besides inhibitory effects on the cilia there are excitatory ones. The 8 comb rows are arranged as 4 pairs, each pair sharing one of the 4 nerves from the apical statocyst. The tilted animal is brought back to an even keel by differential action of opposite comb plates (Bauer, 1910). In a quietly swimming and undisturbed animal, waves starting at the apical end of the comb rows are synchronized between members of each pair (Eimer, 1880; Chun, 1880; Child, 1933). This is evidence that the ciliary waves are initiated by nerves at the aboral end of the comb row, but clearly it does not prove that the wave is thereafter propagated along the comb by nerves. That the wave can travel along the comb row in either direction was known to Chun (1880) but at that time did not agree with the known properties of nerves. A cut transversely through a comb row between two comb plates breaks the continuity of the transmission but does not demonstrate what kind of tissue is involved. Isolated parts of the comb rows develop their own pacemakers and beat independently.

Many aspects of the nervous system of *Beroë*, as revealed by vital staining with methylene blue, have been described by Heider (1927). The same structures have been found in *Beroë* and *Pleurobrachia* stained at St. Andrews with methylene blue, confirming the excellence of Heider's account of the following elements.

- (a) Elongated bipolar neurones which stain with methylene blue, and together resemble a nerve-bundle in appearance, run along the bottom of the ciliated groove from the apical organ to the aboral ends of the comb rows. Other fibres continue between the plates of the comb rows and run beneath the groups of elongated cells (polster cells) on which stand the groups of large cilia. A circumscribed nerve strand lying in a ciliated groove between individual comb plates was figured by Chun (1880) for *Eucharis*, so at least in this instance the junction between the nerve and the first comb plate is repeated all along the comb.
- (b) In the superficial epithelium over the whole animal there is a net of multipolar neurones. This net extends into the tissue of the comb rows. Some of these fibres (all of them if this net acts as a single nerve net) must carry the inhibitory excitation which can arrest ciliary activity.

Finally, Korn (1959) has described branching fibres among the ciliated cells from silver-impregnated material, and shows fine terminations reaching to the bases of the cilia.

Methods

Small pieces of the aboral ends of the comb plates of mature *Pleurobrachia pileus* were fixed in 1% osmic acid in sea-water, buffered with veronal to

pH 7.5, to which a little sucrose has been added. Fixation sets a problem, as in all coelenterate tissues, on account of the watery nature of the animals. Sections in araldite were stained with lead acetate by the following method. A small amount of lead acetate is shaken up with a mixture of equal parts of ether and absolute alcohol in a small tube, care being taken to exclude air.

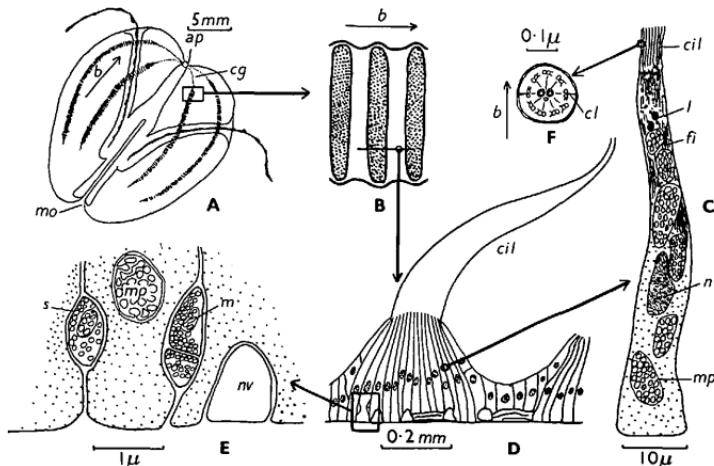


FIG. 1. A series of sketches at different approximate magnifications showing the positions from which the sections of comb plates have been taken. A, whole animal, with a square round the region which was always used. B, 3 comb plates from above, showing the line of sections which is called transverse to the comb plate, longitudinal to the comb row, as normally used in this paper. C, a single polster cell. D, section transverse to the comb plate, as shown in B, showing cell boundaries and the group of cilia. E, detail from the basal regions of the polster cells as shown by the square in D. The cytoplasm of the polster cells is dotted. F, transverse section of a cilium showing the direction of the power stroke in relation to the unpaired fibril (top), the third central fibril, and the compartmenting lamellae. ap, apical organ; b, direction of power beat; cg, ciliated groove from apical organ to comb row; cil, cilia; cl, compartmenting lamellae; fi, fibrils in polster cell; l, lipidal droplet; m, mitochondrion in nerve; mo, mouth; mp, mitochondrion in polster cell; n, nucleus; nv, axon sections; s, synaptic cleft.

The solution is filtered through very closely grained filter paper (Whatman No. 42). The grids, with sections, are soaked in the filtrate for 30 to 60 min. and washed in clean absolute alcohol. If all the solutions are ice cold the sections are less likely to be destroyed by the staining solutions.

Results

The nerve strand of the meridional grooves. The 8 ciliated ectodermal grooves (fig. 1, A) which radiate from the aboral sense organ to the end of the comb rows mark the position of small bundles of bipolar neurones. The neurones readily stain with methylene blue; they then appear individually as

round axons several millimetres long with a bipolar cell body somewhere along their length. These fibres run along the bottom of the furrow in the neighbourhood of ectodermal cells which bear cilia. They stand out as empty round tubes in araldite sections cut at $0.5\ \mu$ and stained with toluidine blue for light microscope studies. That these fibres are nervous is shown structurally by their appearance as typical unsheathed axons under the electron microscope, by their vital staining with methylene blue, and physiologically by the fact that when this bundle is severed the comb no longer initiates a beat at the same time as the neighbouring comb under the influence of the terminal statocyst. A distinction between the definite excitatory and possible ciliary inhibitory fibres in this bundle cannot as yet be made, but all axons look similar and there are no physiological or histological signs of ciliary-inhibiting fibres.

The axons of this nervous strand are remarkable for their empty appearance under the electron microscope. The largest 2 or 3 of them reach $10\ \mu$ in diameter. The bounding membrane is that of the axon only, with no trace of sheathing cells, which are defined as cells creeping along the axon as distinct from cells among which it passes. The axon membrane lies against the mesogloea or other cells or can be in contact with the external sea-water. This is even less specialized than in most coelenterate tissues, where neurones are usually surrounded by epithelial cells or, as in *Hydra*, where they can lie in a groove with a structure corresponding to a mesaxon. There are signs that these neurones may be ciliated. An occasional mitochondrion is situated anywhere in the axoplasm, and usually there is an accumulation of granular material round the inside of the axon membrane. Small vesicles of 30 to $45\ m\mu$, which, in contrast with jellyfish vesicles, never have dense centres or spots, are similar to those which characterize synapses upon effector cells. In the empty axons of the nerve strand they are thinly scattered, but in the thin fibres which run in the deeper parts of the comb plates they are sometimes abundant. This could serve as a basis for distinguishing 2 types of nerve-fibres, or could be no more than a sign that the terminations are near.

Contacts between the axons are numerous since they lie almost in a bundle along the bottom of the groove in the epithelium. Since continuity exists along the nerve strand there must be a transmission of impulses between axons, because in methylene blue preparations none run for more than a fraction of the length of the strand, which is long in *Pleurobrachia*. Histological synapses, as identified by a synaptic cleft and vesicles, have not been found in sections examined with the electron microscope but this is no surety of their absence. However, where neighbouring axons adjoin, their membranes run very close together for long distances. As in the intercalated discs in cardiac muscle the situation is reminiscent of the cleft at synapses, but without synaptic vesicles. Electrical transmission between these axons cannot be excluded, and would explain the anatomical relations. In his review of the ultrastructure of arthropod neurones, Trujillo-Cenóz (1962) suggests that long lateral contacts between axons may be sites of interaction, although without vesicles.

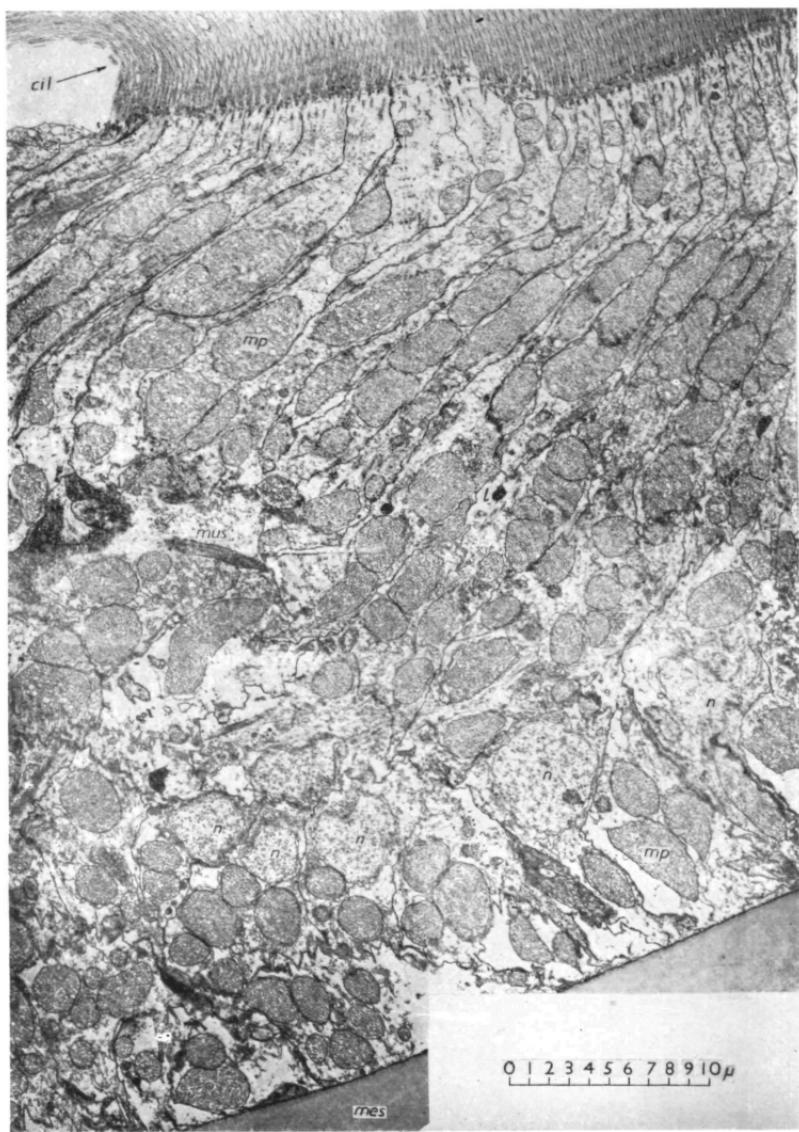


FIG. 2
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The ciliated cells of the comb. The great cilia of the combs are borne at the ends of long cells (called polster cells in all the classical works). Each group of these cells forms a raised ridge transversely across the comb plate, separated from neighbouring groups in the comb by an area of thin ectoderm in *Beroë* and *Pleurobrachia* (fig. 1, A to D). Details of the polster cells, without the nerve-fibres, are illustrated by Hertwig (1880), Samassa (1892), and Schneider (1892). In staining more darkly with lead the cytoplasm of the cells of the un-specialized epithelium of the comb between the comb plates contrasts strongly with the polster cells, which have a striking appearance with their abundant large mitochondria and clear cytoplasm. The polster cells contain ciliary roots, longitudinal cytoplasmic fibres, a nucleus, many large mitochondria, and numerous small round empty vesicles of 30 to 100 m μ diameter, but apart from these the cytoplasm is relatively clear, looking rather like axoplasm. The normal epithelial cells creep up the sides of the marginal polster cells around each comb plate. The polster cells, with specialized cytoplasm, form a separate group, sharply defined from the surrounding epithelium, and only their ciliated ends are exposed to the sea-water.

The polster cells appear to be essentially all identical. They are up to 100 μ long and 6 to 12 μ wide, being closely packed with no other types of cells among them except the processes interpreted as being of muscle- or nerve-fibres (fig. 2). The membrane at the surface of each cell bears from 15 to 50 cilia, as described below. The cells are remarkable for their inclusions. The general appearance is shown in figs. 2, 3, and 4.

Large oval mitochondria, ranging in smallest diameter from 1 μ to 4 μ , and up to 6 μ long, occupy most of the contents of each polster cell. Each mitochondrion has a double outer membrane and is filled with a mass of tubules, which in section could be mistaken for vesicles. The tubules range in diameter from 100 to 200 m μ . The tubules are invaginations of the inner mitochondrial wall. The mitochondria are distributed both above and below the nucleus, which lies near the middle of the cell (fig. 2). The great number of mitochondria suggests a high metabolic activity and presumably the polster cells supply the energy for the continual activity of their cilia. The largest mitochondria tend to be in the central region of the cell, where sometimes a single one may be up to 5 μ wide and 6 μ long and occupy the whole of the width of the cell (figs. 2, 4). A cycle of formation, growth, and disintegration of mitochondria is suggested by their appearance as we look along the cell from the nucleus towards the distal end bearing the cilia. The very large mitochondria do not occur within 10 μ of the ciliated membrane. The cytoplasm here contains, instead, small mitochondria with sparse tubules within them, and numerous vesicles of 100 to 200 m μ diameter abound in the cytoplasm. From their size and the appearance of their bounding membrane these

FIG. 2 (plate). Vertical section through a comb plate in line with the length of the comb, as in fig. 1, B. *cil.*, cilia; *l.*, lipidal drop; *mp*, mitochondrion of a polster cell; *mes*, mesogloea; *mus*, muscle-fibre branch; *n*, nucleus. Each small division on the scale is 1 μ .

vesicles could be the remains of the tubules of disintegrated mitochondria, which sometimes appear to be shedding vesicles on the ends pointing towards the cilia. Some of the vesicles appear to be extruded from the cell in the region of the outer membrane between the cilia, as described below, and others are found within the membranes of the cilia, as figured by Afzelius (1961).

The nuclei of the polster cells are not remarkable. About $10\ \mu$ long by $5\ \mu$ wide they lie at about the centre of the cell. There is a dense nucleolus; the nuclear membrane is double, with pores which have a structure across their opening, unlike the wide open nuclear pores of *Hydromedusae*. The nucleus is frequently indented by a mitochondrion lying against its membrane. In these circumstances the density of the staining in the cleft between the 2 structures and on either side suggests a region of especially high activity.

Electron-dense round bodies in the cytoplasm are frequently distributed about $10\ \mu$ from the ciliated membrane in separate cells but grouped together in the region which limits the large mitochondria. They range from 0.1 to $2.0\ \mu$ usually with a bounding membrane. They are interpreted as lipid food reserves.

Fibrils running longitudinally in the cytoplasm of the polster cells are more numerous towards the bases of the cilia, towards the roots of which many of them are directed. They resemble neurofilaments and are quite different from the neurotubules of jellyfish neurones (Horridge and Mackay, 1962). Their appearance is shown in fig. 4. Some of these fibres, at least in the region near the cilia, are a part of the root structure of the cilia (fig. 3, B). Neurotubules similar to those in jellyfish neurones have been observed in polster cells and in the nerve-fibres.

The cilia themselves are described by Afzelius (1961). They are crowded together so that their enveloping membranes are pressed side by side. Crowding is maintained right to the edge of the group of cilia, with no tailing off (fig. 4). The direction of the beat is at right angles to the line through the central pair of fibrils with the power stroke in the direction of the unpaired fibril (fig. 1, F). Each polster cell has from 15 to 50 cilia, depending on the area of its exposed end. The rhomboidal pattern of close packing of cilia runs straight across the pattern of cells as shown by the straight lines in fig. 3, A.

The bases of the cilia are relatively simple structures and provide no clue as to the control of cilia by each other or by structures within the cell. There are no fibrils connecting the roots of neighbouring cilia, even within a single cell. The only certain point bearing on the mechanism of co-ordination of

FIG. 3 (plate). The peripheral region of the polster cells and the emerging cilia.

A, section grazing the ciliated surface of the cells, going deeper from left to right, showing the arrangement of the cilia, irrespective of the cell boundaries, in ranks parallel to the ink lines.

B, vertical section of the same region which, together with A, shows the loose fibres which are the continuations of root structures. Note the vesicles close to the membrane between the cilia and similar vesicles scattered in the peripheral cytoplasm. cm, cell membrane; f, fibrils associated with the roots of the cilia; r, root structure, in which the 9 but not the central 2 fibrils participate.

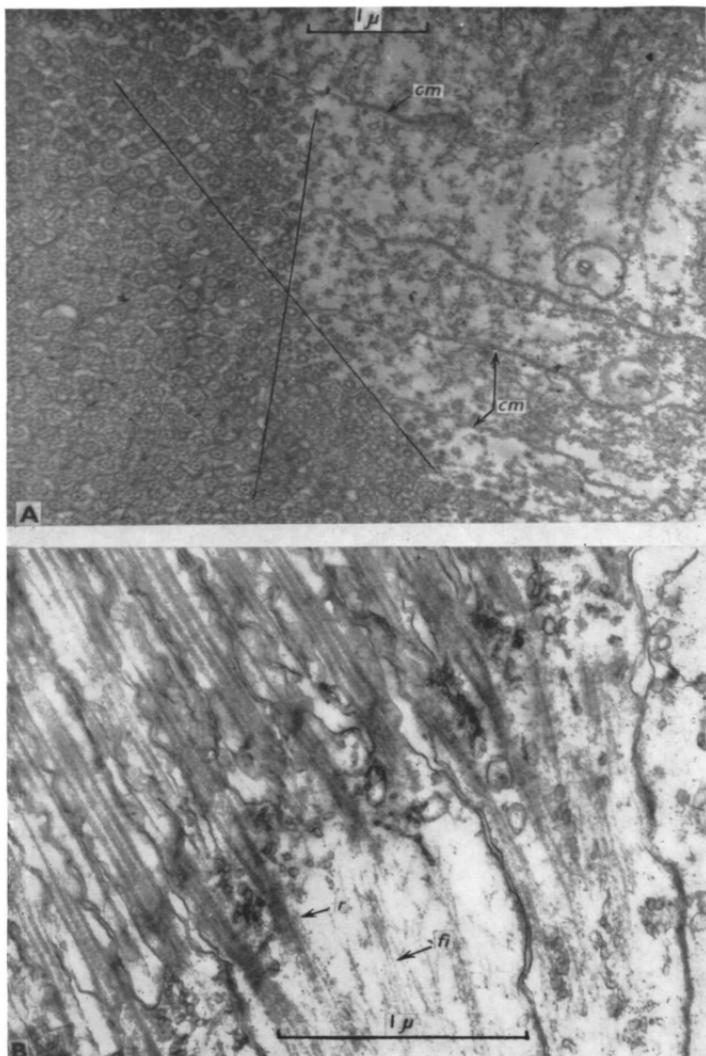


FIG. 3

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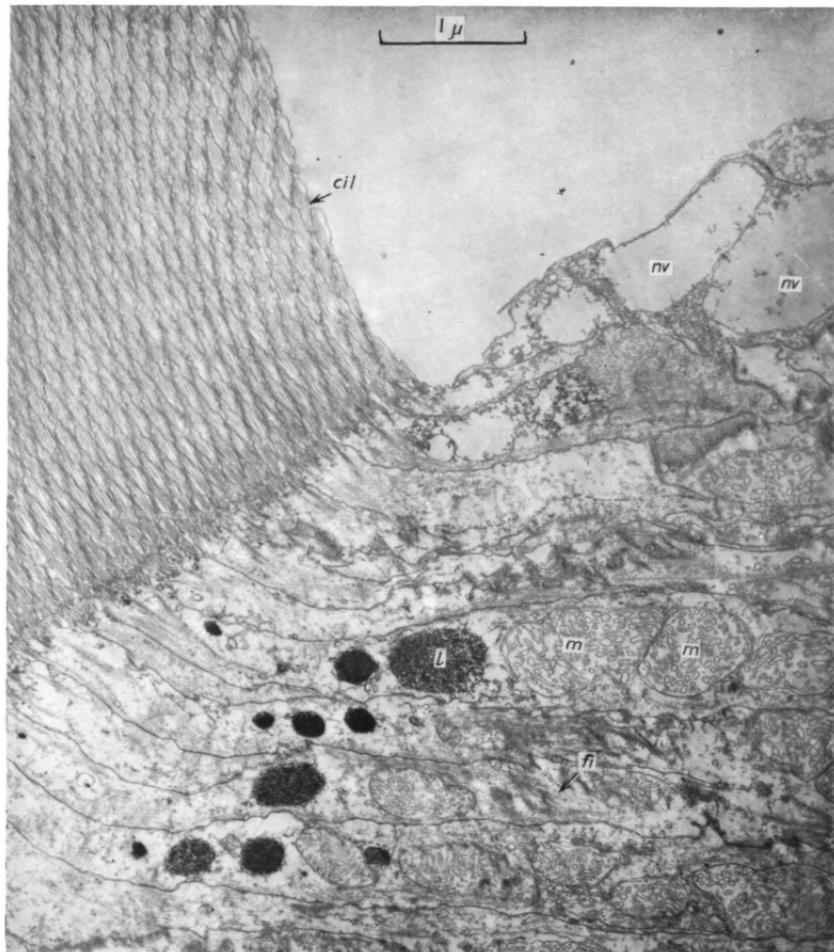


FIG. 4
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cilia is that the nerve endings are far away, usually at the other end of the long cells. The membrane which covers each cilium is continuous with the cell membrane. The inner pair of ciliary fibrils end at about the level where the cilium meets the cell body. The outer ring of 9 fibrils continues for 200 to 500 m μ into the cytoplasm, drawing closer together and becoming less definite in outline, with a short striated region. The 9 run together to form a fibrous mass from which many loose, irregularly arranged fibres emerge on the central side and continue into the cytoplasm almost as far as the nucleus (figs. 3, B; 4).

Nerve-fibres among the ciliated cells. Nerve-fibres of the ciliated groove run into the comb row at its aboral end. A section through the terminal comb plate shows numbers of nerve-fibres which have here the same empty appearance as they have in the groove (fig. 5, A). In longitudinal section these fibres sometimes have vesicles in them and occasional neurotubules but no fibrillae. No typical synapses as recognized by cleft and vesicles have been found between axons of this type or between them and other cells, but their membranes press closely against each other and against the membranes of the basal parts of the ciliated cells. Occasionally a few small vesicles of 30 to 60 m μ diameter are found in these fibres.

A different type of structure interpreted as a section of a nerve-fibre has been found as a result of a search through many sections of comb plates. Between the ciliated polster cells, usually at their basal end, are longitudinal or transverse sections of fine fibres about 0.5 to 1.0 μ in diameter (f in fig. 5, B, C). These are distinguished by having many vesicles and in forming synapses with the ciliated cells. The general appearance of the vesicles in these fibres, in lead-stained sections, is similar to that of nerve fibres in several groups of coelenterates (Horridge and Mackay, 1962).

Synapses on the ciliated cells. A careful scrutiny of sections reveals nerve terminals down to 0.3 μ packed with vesicles of 30 to 45 m μ (fig. 6, A to E). These terminals form structures which closely resemble synapses in other animals. There is a sharp cleft of 10 to 12 m μ against which the vesicles are packed. Frequently in the nerve terminal there is a rather small mitochondrion with few cristae. No special structures are seen on the side of the synapse within the adjoining cytoplasm of the ciliated polster cells. The only feature, besides those mentioned, which helps in the identification of these structures as synapses is their consistency: for this reason a number of examples are illustrated (figs. 6, A to E; 7, A to D). Reasons will be given in the discussion for considering these nerve terminals as the ciliary inhibitory synapses of the diffuse epithelial nerve plexus.

Many of the synapses are double, with sections of 2 adjoining fibres (fig. 7, A to D). Sometimes there are vesicles and a cleft only where the nerve-fibre

FIG. 4 (plate). Typical peripheral ends of the polster cells, shown here at the edge of the group of cilia. No structures resembling nerves run to the bases of the cilia. At the upper right hand corner are sections of 2 typical axons (*nv*) similar to the excitatory axons found in the nearby ciliated groove. *cil*, cilia; *fi*, fibrils in the distal cytoplasm of the polster cells; *l*, lipidal droplet; *m*, mitochondrion; *nv*, sections of axons.

meets the polster cell but sometimes there is the appearance of a typical synapse between the 2 nerve-fibres with vesicles on both sides of the cleft (fig. 7, A, C, D). At present there is no way of deciding whether we have here 2 functionally different fibre terminations, as in sequential synapses of vertebrates (Gray, 1962), whether we have a section through a convoluted ending with the same fibre seen twice in section, or some other unknown relationship. Apart from this, these sections are similar to those with single synapses (fig. 6).

Transmission between ciliated cells. The cilia of a single comb plate all beat at the same time. However, in the absence of any physiological data, nothing is known of the pathways of transmission which normally ensure this co-ordination. Possibilities, between the cilia, are (a) the first cilia to move excite the others of the adjoining cells by mechanical traction forces, (b) transmission is via a chemical or electrical process between the cilia. Bearing on these possibilities is the close contact of the membranes of adjacent cilia and the array of the 'compartmenting lamellae' (Afzelius, 1961; Sleigh, 1962). Between the cell bodies of the ciliated cells there are at least 2 other possible routes of rapid co-ordination, (c) a wave of depolarization across the superficial membrane which is exposed to the sea-water, in which case the walls between the polster cells need play little part, and (d) by transmission between the side walls of the polster cells. Besides these there is a fifth possibility (e) that the polster cells are all separately excited by nerve-fibres. This last is difficult to assess because the number of nerves and especially the number of synapses per polster cell is not known and the excitatory fibres, at least from the ciliated groove, seem not to form obvious synapses.

Within one comb plate the cilia are in contact with each other laterally. Although this may be a sufficient, and perhaps normal, pathway of co-ordination, it is not a necessary one, because when the tufts of cilia are separated or frayed into 2 or more parts down to their base, separated groups of cilia still beat at the same time. Therefore, transmission must be possible within the tissue of the comb plate.

To try to eliminate some of the possibilities and as a search for morphological clues, the walls of the polster cells have been scanned. No large gaps

FIG. 5 (plate). Sections through bases of polster cells adjoining the mesogloea showing the positions of structures interpreted as nerves.

A, the base of a comb plate where the bundle of empty axons of the ciliated groove enters. Empty areas surrounded by membrane are interpreted as excitatory axons.

B, the base of a larger comb plate further along from the end of the comb row. Besides the muscle-fibre and the inclusions such as mitochondria and nucleus typical of polster cells, there are 2 structures interpreted, on different grounds, as nervous. They are the empty fibres (*nv*, as in fig. 3, A) and others (*f*) filled with vesicles. A large example of the latter (*ves*) lies in the centre of the illustration.

C, a region similar to 5, B, showing fibres near the mesogloea in longitudinal section. *f*, fibres, probably nervous, in longitudinal section; *m*, mitochondrion; *mes*, mesogloea; *mus*, muscle; *n*, nucleus; *ms*, structures bounded by a cell membrane, interpreted as sections of axons; *ves*, large structures containing vesicles which are smaller than those in the polster cell, but similar to vesicles which distinguish nerve-fibres, and similar to synaptic vesicles.

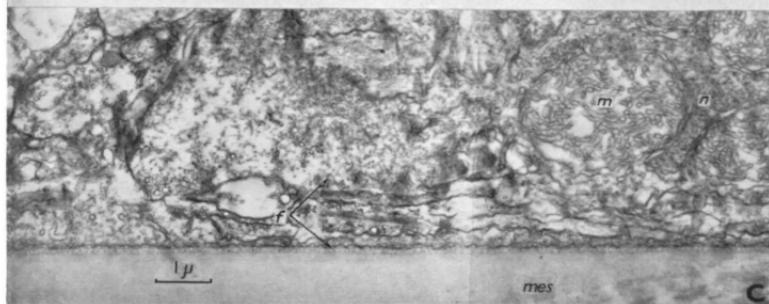
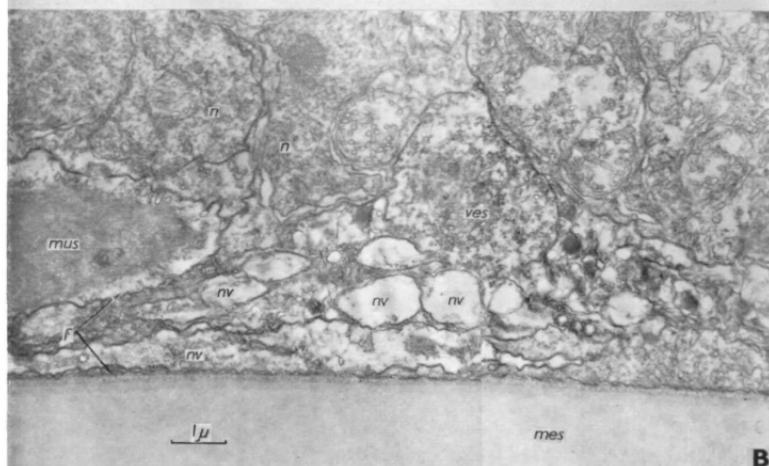
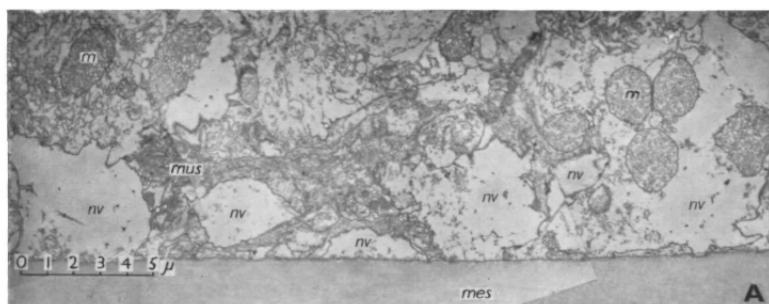


FIG. 5
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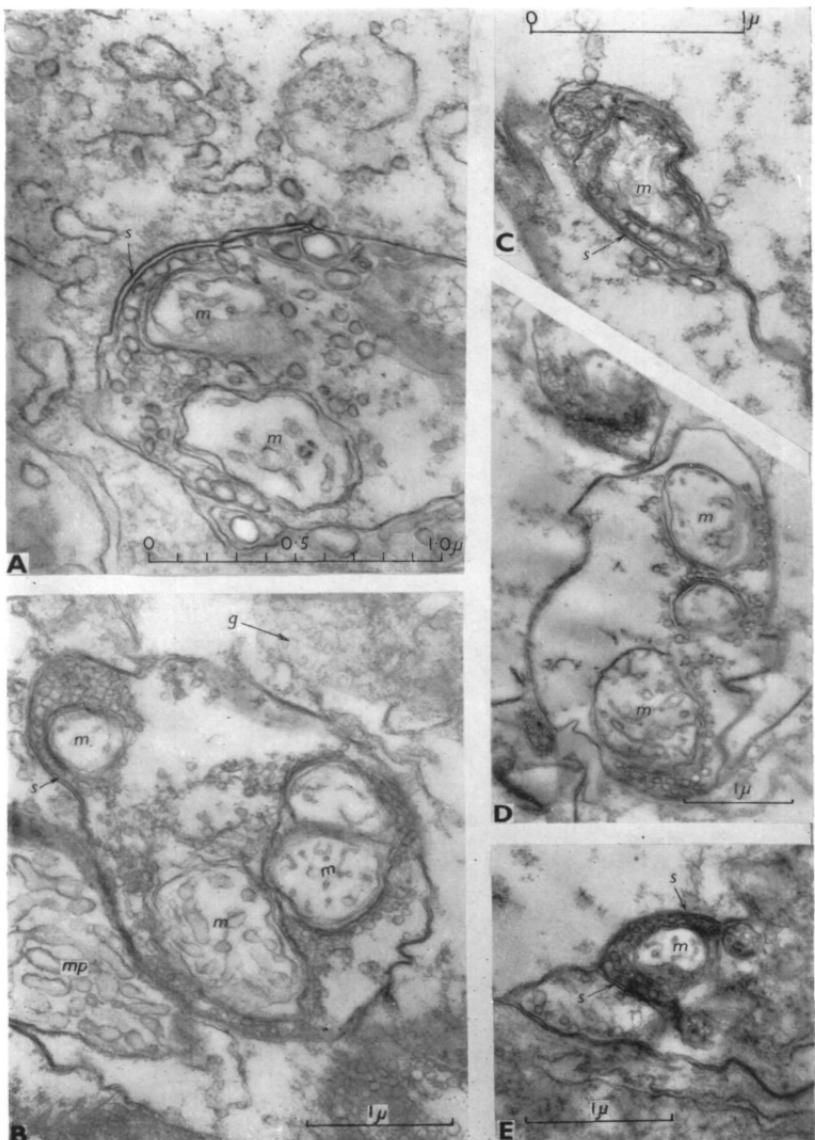


FIG. 6

G. A. HORRIDGE and B. MACKAY

in the walls or signs of anastomosis, such as occur, for example in the endoderm of ctenophores, or in *Hydra* tissues, have appeared. However, the cell walls lie closely apposed together and between them one can find relationships which have some resemblance to synapses (fig. 8). Some of the basal areas of polster cells, adjoining the mesogloea, resemble axons in being clear and relatively free from inclusions. There may well be a continuous gradation between excitatory axons and ciliated cells. Between these clear spaces one can find vesicles of 30 to 50 m μ pressed against the membrane. The membranes of the 2 cells run parallel with a gap of 10 to 15 m μ . This regularity implies that some solid between them is holding the membranes at a constant separation and the width is consistent with that of the cleft of an electrical synapse. Conclusions to these problems can be reached only when electrophysiological micromethods can be applied. At present the problem of transmission between plates along the comb rows cannot be tackled directly either. The velocity of the wave of approximately 1 cm/sec (Sleigh, 1963) is consistent with a variety of theories which are similar to those set out above.

Discussion

The identification of nerve-fibres in ctenophores is not so easy as in jellyfish. Prior philosophical considerations as to how to define the essential attributes of nerve-fibres in fact do not help to establish the criteria for recognition of nerves when we search for them in these relatively simple animals. In practice one must look at the tissue with all the techniques available and then attempt to classify the cells found. In ctenophores Heider (1927) did this on the basis of staining with methylene blue in conjunction with the known directions in which excitation could be propagated. On this basis the classical polyhedral ectodermal net and the long-known fibres of the ciliated grooves clearly justified to be called 'nervous'. However, in and around the comb plates were many cells which stained specifically with methylene blue but which may never be shown to have any of the physiological attributes of nerve-cells. Methylene blue alone is not a reliable indicator, and some of the cells which Heider illustrates as blue are not elongated. Besides this, the polster cells and especially the granules within them stain readily with methylene blue, a fact which may account for some of the blue stained 'nerves' which Heider found in transverse sections of his fixed methylene blue preparations of comb plates. Similarly Korn's illustrations of silver-stained fibres terminating among the polster cells in the region of the cilia are not convincing as illustrations of nerves. Silver deposits can occur on intracellular fibrils or in the spaces between cells. The necessary reservations in accepting results

FIG. 6 (plate). A to E, examples of structures interpreted as sections through nerve terminals containing vesicles, showing similarities to the synapses of higher animals. The synaptic cleft has vesicles on the nerve side; the other cell involved is always a polster cell, with no specialization adjacent to the synapse. The nerve terminals usually contain a few mitochondria bearing few tubular cristae. g, granules arranged in strings; m, mitochondrion in nerve-fibre; mp, mitochondrion in polster cell; s, cleft interpreted as synaptic cleft.

of this kind in coelenterates have been set out by Pantin (1952) and by Batham, Pantin, and Robson (1961) for work with light microscopy.

In electron microscope material, the recognition of nerves in coelenterates and other primitive animals is limited by quite different considerations from those that hold in material stained for the light microscope, but at the same time certain new features become available for their recognition at this ultrastructural level. In addition, the possibility of transmission directly between cells that are not in any way recognizable structurally as nerve-cells must be reconsidered. For jellyfish nerves, Horridge and Mackay (1962) listed as useful features the round empty elongated shape of the axon cylinders, the neurotubules, and the vesicles. In ctenophores, it is the synapses, with a cleft and group of vesicles comparable with those in higher animals, which prove to be the best indicators of nerves. Curiously this is the reverse of the situation for the light microscope, where we look for signs of synapses at the limits of resolution where nerves are already identified.

In ctenophores only 2 criteria of nerves remain. They are (*a*) the finding of rather empty axons which are readily identified in cross-section where they are known on other grounds to occur in the ciliated groove, and (*b*) the finding of synapses on the polster cells and the assumption that these are sections of fibres containing vesicles which are synaptic vesicles and therefore within axons, though not necessarily near their terminations. The axons of the ciliated groove can be followed tentatively into the comb plates by their clear contents which are almost devoid of detail. In this they contrast strongly with epithelial cells and with the distal parts of the polster cells, although there are suggestions that the excitatory nerve-cells of the ciliated groove form a continuous series with the polster cells. Apart from the readily distinguishable muscle-fibres, no other types of cells are known in this region; there is no class of cells comparable to glial or Schwann cells.

A similar lack of criteria perhaps accounts for the failure to find nerve-fibres in electron micrographs of *Hydra* (Hess, 1961). Some of the round empty spaces surrounded by 2 membranes, as found in the lower centre of Hess's fig. 15, could be considered to be sections of nerve-fibres 0.5 to 1.0 μ in diameter lying in a groove in an epithelial cell, but in the absence of vesicles or neurotubules no criteria are available for their recognition as nerves. So far as *Hydra* is concerned, the point to be learned from ctenophores is that

FIG. 7 (plate). Further examples of structures interpreted as sections through nerve terminals at or near synapses. In each of these cases there appear to be 2 or more fibres in close conjunction, recalling the arrangement in sequential synapses in which one fibre synapses with a second fibre at the point where the latter synapses with a third structure.

A, 2 fibres between 2 polster cells.

B, here the apparent line of fibres may in fact be one fibre cut several times. The synapse near the mitochondrion is with a polster cell.

C, the vertical structure 0.5 μ wide down the centre appears to be a nerve-fibre but could be a pleat in the wall of the polster cell on the left.

D, a nerve-fibre runs across the illustration; the synapse is with a polster cell. *m*, mitochondrion in nerve-fibre; *mp*, mitochondrion in polster cell; *p*, polster cell cytoplasm; *s*, cleft of approximately 10 m μ , interpreted as a synaptic cleft.

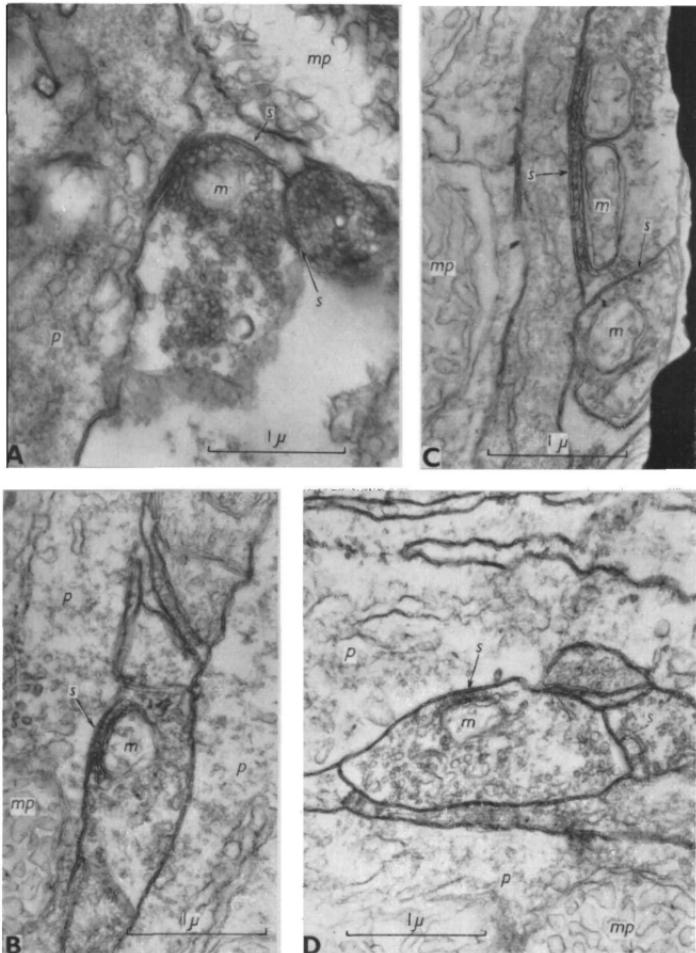


FIG. 7

G. A. HORRIDGE and B. MACKAY

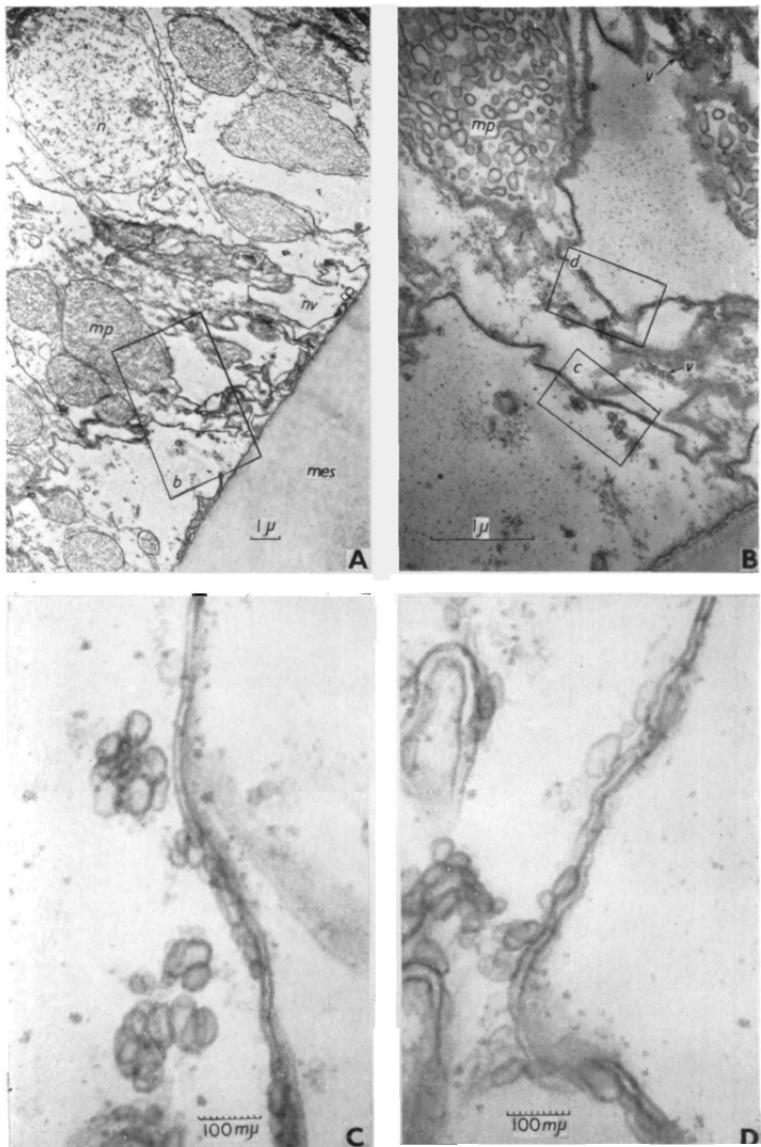


FIG. 8
G. A. HORRIDGE and B. MACKAY

definite nerves, as in the ciliated groove, may have no features which can be used to identify them as nerves under the electron microscope.

The general appearance and contents of the polster cells is different from any other tissue in coelenterates or elsewhere, so far as we are aware. These long thin cells, although crowded with mitochondria, have no trace of endoplasmic reticulum or Golgi complex. Throughout the cell there is a scattering of small granules which frequently seem arranged like beads on a string (fig. 6, b). They resemble granules of the same size which we found in jellyfish neurones, where, however, they were rarely in strings. The polster cells, with their rather clear background cytoplasm, fibrils, and vesicles can be compared with axons, and the most likely conclusion to be drawn from the position of the synapses on them is that they also behave as electrically conducting elements.

The excitatory or inhibitory nature of the synapses which are described here cannot be decided for lack of experimental evidence. One theoretical consideration suggests that most if not all of them may be inhibitory. The excitatory wave is normally propagated along the comb yet any part of the comb can act as a pacemaker in isolation. If not excited by its neighbouring region, any part will develop its own wave of ciliary beat, *unless inhibited*. Therefore, the general inhibitory mechanism must spread to all possible pacemakers, and inhibitory endings must be about as abundant as potential pacemakers. The pacemakers could be the polster cells or the axons interpreted as excitatory; however, the synapses in question occur on the polster cells and not on the excitatory axons. Following the general conclusions for other ciliated cells therefore, we suppose that the polster cells are their own pacemakers; each one may have an inhibitory synapse upon it. This would account for the observed density of synapses. There is an unnecessarily complicated alternative possibility, that some of the 2-fibre complexes shown in fig. 7 represent sequential synapses of a nerve-fibre on a second nerve fibre which in turn synapses on the polster cell. Two nerve-fibres together appear in about one-half of the synapses encountered.

With cilia at the top of a long narrow cell, and the synapses 50 to 100 μ away near the mesogloal end of the cell, one might well ask how the nerves can control the beat of cilia, causing the characteristically rapid inhibition of the beat. In the absence of direct experimental evidence on these cells, the work of Kinoshita and his pupils on the relation between the membrane

FIG. 8 (plate). Cell boundaries as possible excitatory pathways at the bases of the polster cells in a region where excitatory axons from the ciliated groove enter the comb.

A, low power view similar to those in fig. 5, A, showing the polster cells reaching to the mesogloea and a structure (*nv*) which could be classified as a section through an axon.

B, the region of the small square *b* at higher magnification.

C and D, higher power magnifications of the areas which are marked by squares in B, showing vesicles at cell membranes which are separated at a constant distance of about 10 m μ . *b*, *c*, *d*, areas magnified in B, C, and D; *mes*, mesogloea; *mp*, mitochondrion of polster cell; *n*, nucleus; *nv*, structure resembling a section of an axon from the ciliated groove; *v*, vesicles, in otherwise clear cytoplasm, resembling synaptic vesicles.

potential and the ciliary beat in large protozoans provides the nearest relevant concepts (Naitoh, 1958). Change in membrane potential can control the direction of beat of cilia in *Opalina*. Wherever studied elsewhere, the rapid effect synapses have on their target cells is characteristically a change in membrane potential and the mechanism of the inhibitory endings could lie in the suppression of transmission along the polster cells, which in several ways resemble coelenterate nerve-cells.

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