The Neuro-muscular mechanism of the gill of Pecten.

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With Plates 16–18 and 4 Text-figures.

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The gills of Pecten have been the subject of much study, their structure being particularly described by Ridewood (1903), Kellogg (1915), and Dakin (1909). However, there are structures of profound physiological importance present in the gills which apparently have so far remained unnoticed, while data are lacking concerning the neuromuscular mechanism which leads to a definite and orderly distribution of food.
material on the gill surfaces. Further, there are only casual references to the sensory reactions of the gill, without which an adequate understanding of their function is impossible. My own observations disagree with those of Kellogg (1915), who writes, 'Extensive movements of the gills of Yoldia have been described by Drew (1899) and the writer (1890) in which organs there are well-developed muscles, but in the Pecten gill and others also capable of extensive movements such muscles are absent.'

A preliminary survey of the living gill suggested the existence of a neuromuscular mechanism more complex than is indicated from known histological structure. The present work constitutes an attempt to analyse the behaviour of the gill and to correlate this with its histology.

The problem was suggested to me by Mr. Gray. The earlier part of the work was carried out under him and later under Professor Gardiner. To both these gentlemen are due my acknowledgements for their kindly aid and criticisms, and I wish to express my thanks to the University for allowing me to occupy their table at the Marine Biological Station at Plymouth during part of a summer. My thanks are also due to Dr. Allen (Director), Dr. Orton, and other members of the staff for their courtesies to me.

A. Physiology.

(a) Responses of the Gill in situ.

Pecten exhibits a very simple type of reflex activity, the animal responding to all forms of stimulation by the snapping of its shell valves. The response is purposeful, often resulting in the ejection of large quantities of food matter and also of waste material. If the branchial nerve or the palial nerve of the mantle is stimulated, the gills respond by shortening their long axis; they are drawn forwards by the contraction of the ctenidial muscle. When the stimulation is vigorous, the immediate diminution in the extent of the gill is about one-third its original length; the foot at the same time is retracted and only subsequently does the adductor-muscle contract.
This sequence of events is observed by removing one of the shell valves, leaving the animal under water and observing the gills and the foot. After the branchial-adductor cycle, recovery is immediate; the adductor-muscle relaxes first, followed immediately by the relaxation of the gill-muscles.

Text-fig. 1.

Record of response to gentle stimulation on the mantle, resulting in the branchial-adductor cycle. For explanation see text.

A kymographic record of the movements of the gill and adductor-muscle is given (Text-fig. 1). The lower curve represents the gill, the upper the adductor.

The whole cycle can be induced by stimulating either the branchial nerve or the sensory nerves of the mantle (via the palial network).

There is, on the mantle, a well-organized arrangement of excitability; the regions which are particularly sensitive are the anterior and posterior regions of the mantle, where a gentle stimulation calls forth the branchial-adductor cycle; the faeces
and food particles that fall on the mantle are carried by the ciliary tracts on the mantle to the anterior and posterior sensitive regions, where they furnish the mechanical stimulation for this response. If the main branchial nerve is cut (Text-fig. 3 x), the adductor cycle can be induced, but there is no movement of the gills.

This response of the animal is a very specialized feature, and is an extremely important factor for the protection of the gills. This is manifest in the arrangement of the muscular and nervous tissues, and is a necessary consequence of the functional activity of these elements.

(b) Responses of the Excised Gill.

Data in connexion with the sensory physiology of the gills of Pecten are extremely meagre, whereas their functions can only be understood by a thorough analysis of the various movements and sensory reactions of both the gills and the palps, and the relation of these important properties to their histological structure.

The gills of Pecten maximus exhibit incessant contractions which seem co-ordinated in the case of the demibranchs on each side. The extreme sensitiveness of the gills is remarkable, for these structures are capable of responding not only independently of the brain and visceral ganglia, but also when removed from and quite independent of the body. When they are separated from the body they continue to exhibit great sensitivity and respond for two or three days after their removal; they resemble, in this respect, the tentacles of Actinians (Parker, 1917), which when separated from the body of the parent continue to carry out their various activities.

If the gill is cut vertically along its length into several pieces and left under a current of sea-water, each piece continues to respond like the entire gill in all details just as under normal circumstances; all the different movements being reproduced by the excised pieces. This fact suggests that the highly co-ordinated movements of each ctenidium or each piece of gill
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depends on a local mechanism and does not require the interaction of the centralized nervous system. Such activity is exhibited either by muscles which act independently, or under the influence of localized nervous mechanism. In the Pecten gill it is almost certainly due to the latter cause, in view of the intimate relationship of muscular and nervous tissue. Since Englemann (1869) published his work on the Rabbit's ureter, the phenomenon of rhythmical spontaneous movement has been observed in a large number of smooth muscle preparations. Contraction of smooth muscle by direct stimulation is quite common. Lewis and Lewis (1917) demonstrated this in the amnion of the chick embryo. The smooth muscle-cells undergo contraction as early as the fourth day, when there is no nerve supply whatever.

Burrows (1912) showed that very small pieces of heart-muscle from embryos of the chick continued to beat in blood-plasma for as long as thirty days, and the cells in this mass divided and separated from the rest of the tissue and beat rhythmically. In the case of the vertebrate heart, it has been recognized that though the heart-beat is inhibited and accelerated by nerve impulses, the maintenance of its rhythm is independent of its nerves.

The movements of the Pecten gill persist for several hours even after they have been separated from the body and cut vertically into pieces. Such autonomous organs are generally regarded as possessing a nerve-net, that is, a tissue in which there is protoplasmic continuity between the nerve-fibres and nerve-cells; this property is associated with the power to conduct impulses in all directions. Bethe (1908), who investigated Rhizostoma, described elongated ganglion-cells with several nerve-fibres which anastomose with the processes of neighbouring cells. Parker writes: 'As a result of the intimate relation usually existing between the nerve-net and the muscles that it controls, most organs that are provided with this type of neuromuscular organization exhibit an extreme degree of autonomy. This is perhaps one of the most striking features associated with the nerve-net. It is well illustrated by such an organ as the
tentacle of the sea anemone, the autonomy of which was long ago recognized by von Heider (1897).

Another portion of the vertebrate body that exhibits autonomy and at the same time possesses a nerve-net is the digestive tube, especially the small intestine, between the two muscular coats of which is a network of nerve-fibrils, i.e. the plexus of Auerbach. If a portion of the gut-wall is separated into two parts, that portion which includes the myenteric plexus only will respond.

(c) Response of the Gills to Mechanical Stimulation.

1. Concertina action of gill lamellae following mechanical stimulation.

If the gill is stimulated mechanically either by touch or by the presence of a few particles of carmine, two very distinct reactions follow.

(a) If the frontal surface of the gill is lightly touched with a needle, or if a few particles of carmine are placed on its surface, the two lamellae move towards each other (Text-fig. 2). I shall term this the shutting response. The extent of this reaction is apparently proportional to the strength of the stimulus. If the latter is strong the response is instantaneous, the lamellae moving towards each other very actively, while the contraction extends to the filaments in both directions along the gill. If the stimulus is weak only the filaments directly affected are involved.

(b) If a pinch of carmine particles is placed on a lamella, a variation of response occurs. The lamellae instead of moving towards one another first separate slightly further, so that the positive reaction changes to negative; evidently in this case the muscles are differently excited to produce this reaction. This separation of the lamellae is referred to as the opening response, and both the opening and shutting responses which go on quite regularly in nature as a result of stimulation by food particles is described as the 'concertina action'.

A point of special importance is the fact that it is usually the
lamella which is not touched which moves towards the one stimulated. This type of response calls to mind the responses of the siphons (Hecht, 1918), where stimulation of the inside of one siphon results in the closure of the other, the stimulated siphon remaining wide open while the sphincter of the other siphon is called into play (crossed responses).

The most sensitive parts of the gills are those which correspond to the distribution of the main nerve-trunk, i.e. the branchial nerve. The response of the gill to mechanical stimulation is not impaired by severing the connexions with the brain or by cutting the gill vertically into pieces, but if the branchial nerve is cut away, stimulation fails to call forth the 'concertina action'. This is further seen in the extreme anterior portion of the gill which is devoid of the main branchial nerve and is the least excitable portion. When the gill is divided into two parts by a cut at Y (Text-fig. 3), the part A shows no 'concertina action' on stimulation, whereas the part B shows these movements as before.

2. Flapping action of the individual principal filaments.

Besides the 'concertina action' described above, the gill lamellae show a curious flapping action which is not dependent on the main nerve-trunk; excision of the main branchial nerves does not bring about a cessation of this response. In such cases the 'concertina action' ceases.

The flapping movements are due entirely to the principal filaments. Isolated principal filaments when examined under the binocular microscope, being gently touched with a fine glass tube, respond by twisting themselves and moving their sides up and down at right angles to their length like the flapping movements of a bird's wing. The movement is powerful,
characteristic, and the response immediate; the ciliary discs are the most active regions and if to the principal filament are attached any ordinary filaments they also are carried up and down mechanically. When ordinary filaments, cut away from the principal filaments, are stimulated, there is no response. It is therefore clear that the movement of these intervening filaments in the entire gill is mechanical, as the removal of principal filaments render the ordinary filaments motionless.

It matters little whether the stimulus is applied at the ends, in the middle, or near the sides, the response is immediate and the duration of the response extends for several seconds beyond that of the stimulus. Such isolated principal filaments continue to show activity for several hours and are capable of being stimulated by agitation and by drops of water which are allowed to fall upon them. In an attached gill very weak stimuli will call forth this response. If the stimulus is slightly stronger the 'concertina action' comes into play.

I have already mentioned that severing the main branchial nerve produces no appreciable effect on the excitability of the principal filaments; this means that, like the tentacles of Metridium, they have within their structure a nervous mechanism for an unusual degree of autonomy.

In the principal filaments this characteristic is dependent on the presence of nerve-cells on either side of the chitinous tube (n, fig. 2, Pl. 16). On the application of methylene-blue these cells present a picture that looks very much like a nerve-net; nothing, however, can definitely be said regarding the relation of these to one another, because in my very rare successful preparations the methylene-blue did not sufficiently differentiate the various elements as to render the form and arrangement of the cells obvious; moreover, such preparations can be watched only for a few minutes, and it is not possible to say whether the cells with their branches form an intimate anastomosis like that figured by Bethe (1903). Another difficulty in such cases is to decide which of the processes arising from a nerve-cell is the axone. There is in connexion with the stratum of nerve-cells on the gill-axis, a felt of very fine fibres which can easily be
followed (fig. 3, Pl. 16); these, in some cases, are very closely applied to the axones of the bipolar and multipolar cells. In such cases it becomes impossible to decide whether there is protoplasmic continuity or merely physical contact. Direct tests of the transmission of nervous impulses could be carried out, because of the form and structural modifications of the principal filaments. In the functioning gill the movement of the filaments vary in the different parts of its length. In some regions the gill concertinas its lamellae, whilst in others it shows flapping movements of its principal filaments. The full movements appear irregular and quite devoid of rhythm, being in reality dependent on the nature and size and on the position of the stimulating bodies either naturally carried into the mantle chambers or artificially set on the gills during the experiments.

Different species of Pecten manifest different degrees of sensitiveness to touch stimuli which in the case of P. maximus is exceedingly high and in P. tigrinus is very low.

(d) Responses of the Gill to Chemical Stimulation.

Dakin (1910) performed experiments on Pecten with osphradium destroyed; but, as the extent of the structure was not then well known, and since in his experiments he failed to remove the entire organs, it is possible that the responses he observed misled him in his interpretation. Experiments of very simple nature were performed by me to test the sensitiveness of the gills with a number of substances, such as alcohol, clove-oil, picric acid, hydrochloric acid. Weak solutions of these were discharged through a fine capillary pipette into the various regions of the gills. On the application of these in the region of the osphradial ridge there was a slight mechanical disturbance; other parts of the gills are very much less sensitive to relatively stronger solutions. Sea-water and star-fish extract discharged into the various regions did not induce any noticeable response. It is evident from these experiments that the gill is sensitive to chemical stimulation and by means of the osphradium is capable of detecting slight changes in the surrounding medium, or, like the olfactory organs of the fish, serving to test
the chemical nature of the water that passes over it, relates the organism to its environment.

It might be mentioned that the continuation of the osphradium on the gill-axis on each side seems anatomically to be well adapted to receive such stimuli because the gills are the first structures which come into direct contact with the water-current flowing either through the gills or over them.

B. HISTOLOGY.

The reactions of the gill, described above, lead to the conclusion that there must exist nervous and muscular elements which have escaped the notice of previous observers. The behaviour of the living gill points to the presence of sensory cells on the ctenidial axis and on the frontal surface of the gill. These elements must be associated with muscular elements responsible for the movement of the gills themselves, and at the same time must be associated with the mechanism responsible for the adductor-muscle cycle. In addition to this localized branchial complex, there must be a nervous connexion between the mantle and the gills whereby stimulation of the former induces a contraction of the ctenidial axis.

The following species were examined histologically: P. maximus, P. opercularis, and P. tigrinus. Most of the histological and experimental observations were confined to the first species on account of its large size.

In working out the histological details many different methods were employed, but no particular method could be singled out as being the best; many were entirely unsuccessful.

Most of the well-known fixatives were employed and after a trial were discarded in favour of a modification of Bouin's (Duboscq) fluid which was made up as follows:

Sat. sol. picric acid in 70 per cent. alcohol . 2 parts.
Sat. sol. corrosive sublimate . . . 1 part.
40 per cent. formalin . . . . 1 part.
Glacial acetic acid . . . . . 1 part.

The well-known silver and gold methods were also tried in
an attempt to demonstrate the finer fibrillar endings, but the results were not good.

(a) The Ctenidial Axis.

The ctenidial axis is clearly built up of muscular and connective tissue; on the outside is the usual epidermis formed of a single layer of cells. In this layer sensory cells occur in very large numbers, the cells differing from the ordinary epithelial cells in that they are slightly larger and possess a bundle of fine cilia much longer than the cilia of the ordinary epithelium. These sensory cells, at their deeper ends, are connected with nerve-fibres in the nervous layer which is well differentiated below the epithelium on both sides of the axis. In this layer are found scattered at frequent intervals large branching nerve-cells (fig. 3, Pl. 16) which are spindle-shaped, bipolar, and multipolar; the nerve elements are clearly differentiated from the other tissues by the very large size of their cell-bodies and by their large nuclei. Beneath the epidermis the nerve-fibres can readily be followed in frozen sections stained by the iron haematoxylin method; the fibres are fairly distinct and run for some distance and seem to anastomose in places. The different methods of methylene-blue adopted proved more or less unsatisfactory. Nissl's-blue stained the nerve-cells fairly well and also the fibres. The presence of a nerve-plexus and of the stratum of ganglion-cells is clearly established.

Fragments of ctenidial axis, like pieces of excised gill, may be kept alive for several hours, and when stimulated continue to show slight muscular contractions and twisting. It would appear that when the sensory cells are stimulated, the impulse is transmitted to the ganglion-cells which, as seen later, I have reason to believe directly excite the muscle to action. The observed sensitivity of the ctenidial axis to direct stimulation is thus directly associated with the presence of a localized receptor mechanism.

(b) The Gill Filaments.

As is well known, the principal filaments are connected to their neighbours along the lamellae by interfilamentar junctions, as
also are the ordinary filaments with one another; these junctions in the three species described take the form of discs provided with stiff cilia (c.d., fig. 2, Pl. 16). Besides these ciliated discs, especially in P. opercularis, the ordinary filaments on either side of the principal filaments possess a ridge appearing in section as a spur turned towards the principal filaments. The principal filaments themselves possess lateral extensions (figs. 5, 6, and 7, Pl. 17), so that the whole system forms accessory interlocking arrangement and may be looked upon as an interesting mechanical device by means of which the flapping extensions on the principal filaments fit into the groove formed by the spur. As already pointed out, the principal filaments are extremely active and are responsible for what is described as the 'flapping' movement. It is therefore reasonable to expect in this region of great activity an additional device which, when the very active principal filaments are moving up and down, causes the extensions to slip into the groove and so prevents detachment of the filaments. The same mechanism also transmits movement to the inert ordinary filaments.

Lining the chitinous tube of each gill filament there is a layer of endothelial cells (e.). The previous accounts of the histology, so far as they relate to this layer, reveal a number of contradictory statements and opinions. Its presence has been recorded by a number of authors in different Lamellibranchs. Thus Kellogg noted a definite lining in Pecten and also in a number of forms, whereas Pelseneer, Janssens, Ridewood, and Dakin deny the presence of a continuous endothelium.

My material fixed with Bouin's fluid and stained with iron haematoxylin, or Dobell's methyl-blue-eosin, shows very clearly the endothelial lining as a definite and continuous layer in all the species of Pecten. With methyl-blue-eosin the chitin stains blue and the endothelial lining, as well as the intra-filamentar septum red, and the nuclei of the endothelium stand out distinctly from the red corpuscles.

In connexion with the principal filaments are developed the highly vascularized respiratory expansions (r.e.p., Text-fig. 4 and fig. 7, Pl. 17) to facilitate the absorption of oxygen by the
blood-stream. For a detailed description of these structures the reader is referred to Dakin's monograph.

(c) Branchial Musculature.

The histology of the muscular system in molluscs has received considerable attention; reference to the literature shows that opinion is divided with regard to the presence of cross striation of fibres. Von Ihering (1878) and Kellogg (1890, 1892) record the presence of cross striation in the fibres of adductor-muscles. Fol (1888) and Roule (1888) deny the existence of striated muscle-fibres, and, according to them, only smooth muscle-fibres are bound in the molluscs. Dakin (1909) drew attention to the striped muscles in the mantle edge of \textit{P. jacobaeus} and \textit{P. opercularis}, where there are transverse striations as in the adductor-muscle of \textit{Pecten}. No reference is made to the muscle-fibres that move the filaments. It is indeed stated by Kellogg that in the gill of \textit{Pecten}, and of other Lamellibranchs also capable of extensive movements, muscles are absent. Dakin draws attention to the interesting fact that mantle-muscles which show obvious striations are engaged in rapid movement, which is also organized and related to the closing and opening of the shell in swimming. To these may be added the longitudinal ctenidial muscles (Text-fig. 4, \textit{l.c.m.}) as another case of connexion between striation and rapid contraction and relaxation, but, strange to say, such striated fibres were found only in \textit{P. tigrinus} and \textit{P. opercularis}, not occurring so far as I could ascertain in the ctenidial muscles of \textit{P. maximus}.

Besides these muscles there are in relation to the filaments two other sets which bring about rapid movement and these belong to the smooth muscle type.

The difficulty in distinguishing muscles from connective is great, but the functional relation and microchemical reactions leave no doubt as to their muscular nature. The arrangement of the two sets can be best understood by a reference to Text-fig. 4 and fig. 9, Pl. 18, and the fibres shall be referred to as the transverse (\textit{t.m.f.}) and the criss-cross set (\textit{c.c.m.}).
The transverse set runs transversely across the filaments, and its fibres are inserted on the dorsal and outer sides into the chitin of the principal filaments.

The criss-cross set lies below the filaments, and is also inserted into the chitin of the principal filaments; but lower down to the inner sides, as their name indicates, they run in a criss-cross manner.

The lamellae respond by moving towards each other; these movements characterize just such a group as those described later, and such a function is closely indicated by the direction of the fibres and their relation to the chitinous filaments. These muscles can easily be seen in sections, but the reconstruction of the course they take is a difficult matter, because during fixation the lamellae undergo some amount of distortion.

The functional relation is somewhat as follows. The lower criss-cross fibres by their contraction draw the filaments together; in such a condition the dorsally placed fibres are stretched. On the relaxation of the inner set, the mechanical contraction of the transverse muscles causes the lamellae to separate. It is possible to remove the criss-cross muscle-fibres; when this is done with a red-hot needle, the characteristic response disappears and the lamellae begin to gape. These fibres are usually single, and the individual fibres are large. They have a definite arrangement and present a wavy appearance. The contours and forms of the individual fibres as they appear under the microscope suggest both smooth muscle and connective tissue-fibres.

A number of stains were employed to determine the real nature of the fibres. The material used was fixed in corrosive sublimate.

1. Mallory's connective tissue stain.

The white fibres and reticulum of connective tissue stain blue and the elastic fibres yellow in this medium.

Paraffin sections fixed in corrosive sublimate were stained for about ten minutes in a $\frac{1}{4}$ per cent. aqueous solution of acid fuchsin, rinsed in distilled water and treated for two minutes in 1 per cent. solution of phospho-molybdic acid; they were again
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rinsed in distilled water and transferred to the stain for about fifteen minutes, after which they were rapidly dehydrated, cleared, and mounted. After such treatment the white fibrous connective tissue stained light blue and the two sets of fibres in question stained deep red. This would indicate that the fibres are not composed of white fibrous tissue.

The presence of elastic fibres was sought by means of Weigert resorcin-fuchsin stain, in which the elastic fibres should stain dark blue.

Sections were stained overnight in the above mixture. They were then dehydrated quickly, cleared in xylol and mounted. The connective tissue in the ctenidial axis and round the muscle-fibres took up a dark-blue coloration, whereas the fibres in question appeared dull lavender-grey, and did not show any affinity for the stain. This would mean that the fibres are not composed of elastic connective tissue. The control in all these cases were the ctenidial muscles.

Van Gieson’s picro-nigrosin stains muscles yellowish green and connective tissue blue. Sections were therefore stained by this method for twelve hours, then washed in picric alcohol, rapidly dehydrated, cleared, and mounted. The connective tissue stained blue, the fibres and the ctenidial muscles green. The fact that the fibres in the above experiments react in precisely the same manner as the ctenidial muscles when subjected to the treatment of different stains show that they are themselves composed of muscle-fibres.

Van Gieson’s picro-fuchsin stains muscles yellow and connective tissue red. Sections stained in this medium gave the above reaction.

From these reactions and from those already described regarding the functional relation of the fibres, there are definite grounds for believing that the fibres are composed of muscle-tissue. In addition to these muscles there are other muscle-fibres which run into the interfilamentar junctions (m.f., fig. 2, Pl. 16) and also throughout the length of the principal filaments, and it is due to these that the principal filaments exhibit activity.
(d) Nerve Supply.

(i) Palps.—The palps were shown to be innervated by a branch from the cerebral ganglion (Drew, Dakin), but the definite course of this nerve was not worked out, and the arrangement and connexions of nerve-trunks in the region of the mouth proved to be of exceptional interest.

From the anterior end of the cerebro-pleural ganglia arise four nerve-cords. Two of these are large and well developed. The inner one (c.p.c., fig. 1, Pl. 16) is the commissure that joins the cerebral ganglia; while the other well-developed cord arising from the anterior pleural end is the anterior palial nerve (a.p.n.). Between these two trunks arises a small nerve which runs parallel with the commissure as far as the mouth region, where it joins an enlargement which also receives branches both from the dorsal and ventral edges of the mouth. From this enlarged region extends a fairly stout nerve, consisting of nerve-cells and fibres so that nerve-cells are equally distributed along the whole length; this runs just below the junction of the two palps on either side; it sends out branches to the palps and near the anterior neighbourhood of the gills it splits up into several smaller branches, some of which are continued into the gills. The fourth small nerve takes its origin from the pleural end, runs on the outer side of the anterior palial nerve and ends as shown. In one individual an interesting variation occurs: the palp-nerve which arises between the two main nerve-trunks actually branches off from the small outermost nerve near the middle of its length; the two nerves, figured distinctly, are both referred to as palp-nerves (n.p.).

(ii) Gills.—In the gills besides the scattered nerve-cells and the anastomosis of their fibres over the whole organ, there are four main longitudinal trunks (Text-figs. 3 and 4).

The main branchial nerve arises from the visceral ganglia, bends ventrally and posteriorly and enters the ctenidial axis, each nerve supplying the gill of its own side.

1. The branchial nerve gives origin at intervals corresponding to the principal filaments throughout its length to one single
and two pairs of nerves. The single nerve innervates the osphradium. The succeeding pair of nerves run laterally and are continued down the blood-channels on the principal filaments (n.f., fig. 8, Pl. 17). The last pair of nerves run between the ctenidial muscles and outer epithelium and enters into

anastomosis with groups of ganglion-cells which represent the two lateral nerve-trunks.

2. The two lateral nerve-trunks (b.l.n.) run parallel to the main branchial nerve, and consist almost entirely of groups of ganglion-cells; these nerve-trunks are referred to as two large ganglia. Fibres from these are continued down on each side, they turn inwards below the ctenidial muscles and supply the principal filaments (n.f.). Some fibres from these ganglia also seem to enter the ordinary filaments.

The fourth nerve-trunk which arises from the brain is the subsidiary branchial nerve (s.n.). It runs the entire length of the gill and lies below the main nerve. In frozen sections stained by the Bielschowsky's method, fibres from this nerve to the transverse muscle are easily distinguished (s.n.f., fig. 9, Pl. 18). By reason of the presence of nerve-fibres, in sections of ordinary filaments, considerably anterior to the three longitudinal nerves
described above (Text-fig. 2), it seems probable that the subsidiary nerve innervates these filaments as well. The supply from this nerve is scanty as compared with the extensive innervation from the main nerve-trunk.

All attempts to determine the function of the subsidiary branchial nerve yielded, unfortunately, negative results. Of these, the following may be of interest.

1. Immediately the shell was removed and while the gill lamellae were together (that being their usual condition before shell removal), a cut was made at \( x \) (Text-fig. 2) to see what influence the nerve has on the effector system of the gill. On gentle stimulation the lamellae moved apart as rapidly as in the case of the other gill in which the connexions were not broken, neither were the spontaneous contractions which sometimes occur modified in any appreciable manner.

2. From one animal a gill was removed from the body and a comparison of its behaviour with that of the intact gill was made. Both the gills responded to stimulation in a similar manner; they opened and closed in the same amount of time for the first half-hour or so, later the same method of treatment caused the excised gill to open much quicker than the other. The experiment was repeated again on fresh material, but I was not able to obtain any very conclusive evidence for the existence of inhibitory activity.

An attempt was also made to stimulate the nerve in the middle of its length and also near the palps; varying strengths of current were used, but in no case had this any observable effect. While its sensory function cannot be denied, another possibility is that the cilia on the palps and the gills may be under nervous control.

Nelson (1924) in spat oysters describes the rejection of particles as due to reflex erection of the ridges of the palps which bring into action groups of cilia which beat away from the mouth.

On cutting the subsidiary branchial nerve, however, there is no evidence of reversal either on the gills or on the palps, nor does mechanical stimulation alter the direction of the ciliary
stroke. The function of the subsidiary branchial nerve remains, therefore, obscure.

(e) The Osphradium.

A reference to standard literature is sufficient to show that the organ has been extensively studied since its discovery in 1881 by Spengel. It has been the subject of a paper by Dakin (1910), who described the anatomy and considered the structure in a comparative way in a number of Lamellibranchs with a review of the question of innervation. His description of the structure and innervation of the organ as present in the vicinity of the visceral ganglion is in accordance with my observations.

As to the function he writes: 'The experiments made with the osphradia removed were unfortunately not quite conclusive enough. These organs, as described above, lie in close proximity to the visceral ganglia. An attempt was made to remove the sensory epithelium by scraping with a scalpel; however, since the osphradia are unpigmented, it was not possible to see whether any remains of these organs were left. The individuals, however, treated in this way appeared to react to the star-fish pulp, &c., &c. On the whole, I believe, the star-fish pulp was recognized by the mantle edge with its sensory tentacles and any action of the osphradium is very questionable.'

In my sections of the gill axis, the most interesting feature is a continuation of the osphradium in the form of a ridge on the gill axis (Text-fig. 4, o.). Most of the fixatives that I employed brought about shrinkage, distortion, and destruction, as a result, to the naked eye, the osphradium is scarcely visible, but there is never any difficulty in recognizing it under the microscope, as it is easily identified by its elevated nature, in stained or unstained sections. It is a constant organ of the gill axis and for the reasons mentioned above has remained unrecognized so far. I examined this organ by the help of several sets of serial sections in different planes, the osphradium is seen to be continued on either side of the gill axis to the extreme tip of either gill. It occurs in the position described in all the species of Pecten investigated by me, as well as in Glycimeris.
glycimeris. It takes a course parallel to the main branchial nerve; sometimes on a level with it and at others a little below. It is innervated from the main branchial nerve, bundles of delicate nerve-fibres are given off on its side; these arise at intervals and enter the structure at its base (b.n.f., figs. 9 and 10, Pl. 18).

The epithelial cells of the region of the osphradium are more or less columnar and consist of central cells which are somewhat larger than the surrounding cells. They are broader in the
middle, in which portion the nucleus is situated; the basal end is the narrowest region and seems to be continued into a nerve-fibre. At their anterior ends the cells appear to be prolonged into stiff cilia or sensory hairs, which lie below the well-developed cuticle. Such cilia, and to some degree the total structure, can be seen in some regions, but was not demonstrable as a regular feature along the whole length of the organ. List (1902) defines the osphradial area as follows: 'The cells of the osphradial area are to be distinguished by a complete absence of cilia, a distinct marginal cuticle, and an increased depth.' In Pecten, according to my observations, only the last two characteristics are present; there are in addition to the structures described above well-developed sensory cells with their cilia in the osphradial area (s.c., fig. 10, Pl. 18).

Another feature of the osphradium in this position is the presence of bipolar cells. Besides the bundle of nerve-fibres from the main branchial nerve, the osphradium has another bundle of fibres running in the middle throughout its course; these fibres (l.n.f., figs. 10, 11, Pl. 18) run below the epithelium parallel to the main branchial nerve-trunk, amongst its fibres bipolar cells occur (b.c., fig. 11, Pl. 18). Dakin gives a detailed description of the innervation of this organ. It is not my object to repeat Dakin's observations, but an attempt was made to trace to its origin the longitudinal bundle of nerve-fibres without any success; this was not determinable either by dissections or in sections, but they must come either from the brain or from the visceral ganglia.

In this connexion it might be interesting to mention Dakin's view: 'It is quite possible and probable that processes of some of the ganglion-cells concerned in the innervation of the osphradium pass into the cerebral and visceral connectives in addition to those which enter the visceral ganglion, but it is not easy to follow individual fibres the necessary distance in sections, and the evidence appears to show that the visceral ganglia are concerned in the innervation of the osphradium.'
C. Gill Movements and Feeding Habits.

As is well known, there are several ciliary currents on the surface of the gill.

1. A very fast frontal system which runs up the grooves on the principal filaments, along the top edge of the gill, between the palps, to the mouth.

2. A system of slower frontal currents which run down the crests on the ordinary filaments, along the free edge of the gill into the groove at the edge, where the particles are formed into slimy strings; these usually find their way on to the mantle from whence the accumulation is thrown out by the branchial adductor-cycle.

Carmine particles dropped on the gills are thus at once sorted out into two definite streams, one moving towards the base and the other towards the edge. These directions of movement in agreement with the ciliary activity are not always constant, since certain of the larger particles may leave their course, be moved on to the crests and continue their course in the opposite direction. This act of transference being evoked by the presence of the large or more irritating particles on the principal filaments, the impulses due to their presence are transmitted up and down the principal filaments and the flapping movement, which is a very adaptative response, results. It is the most useful movement to get rid of the large particles, and this it evidently accomplishes by throwing them on to the crests of the adjoining filaments; this activity results in a continuous arranging and rearranging of particles. The fine particles, which are more firmly held in the mucus on the principal filaments, continue their course unaltered; whether these would find their way into the mouth depends largely on the activities of the palps.

Finally, under the influence of strong stimuli, the flapping movement is followed by vigorous concertina action; this striking sensory response, resulting in proportionally greater activity, deals with the bulk of material and its primary importance is that it serves as an expelling force.

The downward moving streams of food collect in the grooves
at the edge of the gill and are propelled anteriorly. When the accumulations in this position become fairly large, the gills twitch owing to the mechanical pull of mucus threads, so that the load is dislodged on to the mantle. If the string of mucus by any means persists in sticking to the edge, the response is repeated and a concerted effort seems to be made by the neighbouring filaments which bend in the direction of the stimulating body and try to dislodge it. For a more complete account of the ciliary currents on the gill, reference should be made to Kellogg's paper.

The data presented in the present paper seems to justify the view that the selective feeding mechanism of the gills of Pecten is not solely the result of ciliary activity, but is the result of a highly co-ordinated system in which ciliary, muscular, and nervous elements all play an important role.

The experimental and histological data are clearly in harmony with each other, although no function is as yet assignable to the subsidiary branchial nerve, nor is it possible to demonstrate an exact parallel between the distribution of the diffuse receptor-cells on the gill-surface and the areas which respond to mechanical and other forms of stimulation. On the other hand, each physiological phenomenon has been traced to specific histological elements—and an improvement of technique may, in the future, result in still closer approximation of structural form to physiological activity.

D. Summary.

Experimental.

1. The contraction of the adductor-muscle which follows stimulation of the palial nerve is preceded by a marked contraction of the ctenidial axis, so that the gill contracts before the adductor-muscle becomes active. This movement of the ctenidium is abolished if the main branchial nerve is cut near its origin.

2. The gills of Pecten possess a neuromuscular mechanism which is to some extent independent of the rest of the body, so that excised gills when stimulated react in the same way as an attached gill.
3. The lamellae of the gill possess two distinct types of movement.

(a) When the surface of the gill is stimulated by contact with a glass rod or by carmine particles, the frontal surfaces of the two lamellae approach each other; the movement very often being executed by the lamella which is not actually being stimulated. The lateral extent of these movements (concertina movements) is roughly proportional to the intensity of the stimulus. Such movements normally appear to transfer the bulk of the material on to the mantle. Separation of the main branchial nerve abolishes these movements.

(b) Each principal filament is capable of moving the ordinary filaments to which it is attached. This movement (flapping movement) is due to the movements of the interfilamentar junctions which alternatively move up and down at right angles to their length. This motion is independent of the branchial nerve and can be produced by direct stimulation of very tiny pieces of the individual filaments.

4. The significance of gill movements to feeding habits is discussed. The course of food particles depends on the nature of the stimuli affecting the gill.

Histological.

5. The ctenidial axis and the principal filaments have a stratum of anastomosing nerve-cells which appear to form a true nerve-net comparable to that of the mantle.

6. The gill receives nerve-fibres from two sources, the brain and the visceral ganglion. The subsidiary branchial nerve is a structure hitherto unknown in the molluscan gill; so far its function is unknown. Each gill has four main longitudinal nerve-trunks.

7. The osphradium of the gill has a much more extensive distribution than has hitherto been supposed.

8. Two sets of muscles exist at the base of the gill-filaments,
GILL OF PECTEN

and these are responsible for movements of the lamellae. The muscle-fibres are non-striated.

9. The principal filaments are connected to the ordinary filaments by processes containing true muscle-cells, and by these cells movements of the filaments are effected.

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EXPLANATION OF PLATES 16-18.

LIST OF ABBREVIATIONS.

a., axone; a.f.c., abfrontal cilia; a.p.n., anterior palial nerve; b., blood-corpuscles; b.a.v., branchial afferent vessel; b.c., bipolar cell; b.e.v., branchial efferent vessel; b.l.n., branchial lateral nerve; b.m.n., branchial main nerve; b.m.f., branchial nerve-fibres to osphradium; b.v.p., blood-vessel of principal filament; c., cilia; c.a., ctenidial axis; ch., chitin; ca., cuticle; c.c.m., criss-cross muscle-fibres; c.d., ciliated discs; c.t., connective tissue; c.p.g., cerebro-pleural ganglia; c.p.c., cerebro-pleural commissure; c.p.co., cerebro-pedal connective; c.d.p., exterior labial palp; e., endothelium; f.c., frontal cilia; f.n., filament nerve; i.l.j., inter-lamellar junctions; i.l.p., interior labial palp; i.f.s., intra-filamenter septum; l.c., lateral cilia; l.c.m., lateral ctenidial muscle; l.l., lower lip; l.n.f., longitudinal nerve-fibres; m., mouth; m.c., mucus-cell; m.f., muscle-fibres; n., nerve-cells; n.f., nerve-fibres; n.p., nerve to palps; o., osphradium; o.f., ordinary filament; p.f., principal filament; p.g., pedal ganglia; p.n., pedal nerve; r.e.p., respiratory expansion of principal filament; r.g., right gill; s., spur; s.c., sensory cell; s.n., subsidiary nerve; s.n.f., subsidiary nerve-fibres; t.m.f., transverse muscle-fibres; u.l., upper lip.