The Blood-system in the Serpulimorpha
(Annelida, Polychaeta)

III. Histology

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SUMMARY

1. The histology of the blood-system of Pomatoceros triqueter has been studied in
detail. Comparative observations have been made on the following serpulids and
sabellids: Serpula vermicularis, Hydrodes norvegica, Vermiliopsis infundibulum, Sal-
macina incrustans, Protula intestinum, Apomatus ampulliferus, Spirorbis militaris, S. cor-
rugatus, Sabella spallanzanii, Potamilla sp., and Dasychone lucillana. Living coelomic
capillaries of Sabella have been investigated.

2. All vessels possess a three-layered wall consisting of an endothelium, a skeletal
coat, and a peritoneum containing muscle-fibres which lie transversely to the long
axis of the vessel.

3. The outer wall of the gut sinus had the same three layers. On its inner side the
sinus is bounded by an endothelium lying on a skeletal coat which in most species
is the basement membrane of the gut epithelium. In Sabella and Branchiomma a
muscle coat, with fibres lying transversely to the long axis of the gut, is situated inside
the inner skeletal coat and rests on the basement membrane of the gut epithelium.
The function of these muscles is not known. Longitudinal muscles are found in
the same position in Myxicola.

4. The endothelium of the coelomic capillaries of Sabella, and probably of all serpu-
lids and sabellids, is a syncytial reticulum of cell bodies connected by cytoplasmic
strands.

5. The endothelium of Sabella and Branchiomma, but not of the other species
investigated, contains chloragosome-like globules. In Sabella it also contains iron in
some organic compound.

6. Blood-cells are absent from all serpulids and sabellids investigated.

7. The skeletal coat is linked by fibres with the rest of the internal skeleton of the
body. It is a homogeneous sheet of a substance giving the staining reactions of
collagen. Reticular and elastic fibres are absent from it. When the vessel contracts the
skeletal coat does not change in thickness but is thrown into longitudinal folds.

8. The coelomic capillaries of Sabella have a peritoneum which is apparently syn-
cytial, and in which are muscle-fibres arranged in a wide-meshed reticulum. The
reticulum and the nuclei of the peritoneum can be vitally stained with methylene blue.
The nuclei are sparsely scattered over the surface of the vessel without any special
relation to the fibres of the reticulum.

9. On the larger vessels and on the gut sinus separate muscle-fibres, each with one
nucleus, are present. The peritoneum constitutes a muscle-epithelium. The nucleus
lies in a small membrane of cytoplasm extending along the outer surface of the fibre.

10. The muscle-fibres of the gut sinus are composed of unstriped fibrils. The fibres
of the smaller vessels sometimes show alternating stained and unstained bands of equal
length.

11. On the larger vessels each muscle-fibre apparently contains both striped and unstriped fibrils. The fibre seems to be covered by a thin sheath of striped fibrils covering a central core of unstriped fibrils which are arranged so that the core shows a double-oblique striation.

12. The muscles of the rest of the body are unstriped.

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INTRODUCTION

In the first two papers of this series (Hanson, 1950, a, b) the anatomy of the blood-systems of sabellids and serpulids has been described. The present paper concerns the histology of the blood-system in both families.

Previous work has been reviewed in detail elsewhere (Hanson, 1949). The most recent account is that of Ewer (1941), in which are reported some preliminary observations on the vessels of Sabella pavonina and S. spallanzanii. In the walls of the larger vessels and in the outer wall of the gut sinus Ewer found an endothelium lining a 'structureless' membrane, outside which was a circular muscle coat covered by the peritoneal epithelium. In the smaller vessels, including those of the crown, both the endothelium and the muscle coat appeared to be absent. However, Claparède (1873—S. spallanzanii), Löwe (1878—Spirobranchus), Meyer (1888—Hydroides lunulifera, Protula tubulatrix), Lee (1912—Serpula vermicularis, Hydroides norvegica, Vermiliopsis infundibulum, Pomatoceros triqueter, Salmacina dysteri, and Protula intestinum), and Evenkamp (1931—Laonome kroyeri and Euchone papillosa) all described muscle-fibres in the walls of the vessels in the crown, although Faulkner (1930—Filograna implexa), like Ewer, was unable to find them. According to Faulkner all the vessels of Filograna lack muscles, but in Salmacina and other serpulids Lee (1912) found that the walls of all vessels consist of circular muscle-fibres covered by a layer of cells which he thought might be either a peritoneal epithelium or the cell bodies of the muscle-fibres; an endothelium was absent, and the 'structureless' membrane described by Ewer was not identified. Evenkamp's account (1931) of the vessels of Laonome and Euchone resembles that of Lee, but he found an endothelium.
Material and Methods


The vessels of serpulids are closely surrounded by other tissues, and their walls cannot easily be examined while they are alive. However, some of the vessels of *Sabella* can be used for this purpose. The branchial vessel can be dissected out without much surrounding tissue. The best objects are the numerous naked blind-ending capillaries which project into the coelom from vessels lying on the body wall. Examination of these living capillaries is facilitated by the vital staining of their muscles with methylene blue. The stains made by Schering-Kahlbaum, Grübler, and Gurr have been used with equal success. A one per cent. solution of the stain in distilled water was diluted with sea-water to approximately 0.1 per cent.; it was left for a few hours, and then filtered to remove the slight precipitate that had formed. The capillaries were immersed in this solution.

Material for sectioning was fixed in Duboscq-Brasil, Heidenhain's 'Su-a', Zenker-acetic, or Zenker-formol. Most of the sections were stained with Heidenhain's 'Azan', which is particularly useful for distinguishing muscle-fibres from connective tissue fibres in the walls of the vessels.

Observations

General Histology

The observations in the present account of the histology of the blood-system have largely been made on sections of *Pomatoceros triqueter* and extended by comparative observations on other species. The blind-ending capillaries which project into the coelom of *Sabella spallanzanii* have been used for investigations on living vessels. Before giving a detailed account of the results it is convenient to survey the general histology of the blood-system, i.e. to list the components of the walls and to describe their arrangement.

All the vessels have walls composed of three layers (fig. 1). The middle layer, or skeletal coat, is a continuous sheet of a substance which is probably collagenous. On its inner surface lies a discontinuous endothelium (fig. 4). On its outer surface is the peritoneum, containing muscle-fibres which lie at right angles to the length of the vessel. The nuclei of the peritoneum project from the surface of the vessel. The walls of vessels of all sizes are composed of these
three layers. The thickness of the skeletal coat varies according to the diameter of the vessel: it is thicker in larger than in smaller vessels. The endothelium is of uniform appearance throughout the blood-system. The most important difference between small and large vessels is in the structure of the peritoneum. In the smallest vessels it is apparently a syncytial epithelium containing a network of muscle-fibres. In the large vessels it is a muscle-epithelium.

The outer wall of the gut sinus is like the walls of the larger vessels. On the inner side is an endothelium lying on a skeletal coat. The latter is usually the basement membrane of the gut epithelium (fig. 2). In *Sabella spallanzanii* and *Branchiomma vesiculosum* (and also in *S. pavonina*, according to unpublished observations of Dr. A. Stock) a muscle coat, with fibres lying transversely to the long axis of the gut, is situated inside the inner skeletal coat, and rests on the basement membrane of the gut epithelium (fig. 3). In *Myxicola infundibulum*, as Claparède (1873) first discovered, there is a coat of longitudinal muscles.
FIG. 2. Horizontal section through wall of abdominal gut of Pomatoceros triqueter.

FIG. 3. Diagrammatic drawing of longitudinal section through wall of abdominal gut of Sabella spallanzanii.
in the same position. The skeletal coat and the basement membrane are connected with each other by fibrous strands crossing the muscle coat. Evenkamp (1931) has described a similar coat of circular muscles in *Laonome kroyeri* and *Euchone papillosa*. Possibly other sabellids possess muscles in this position. They are absent in all the serpulids which have been examined. The function of the muscles internal to the sinus is not known. In *Myxicola* they are unusual in being longitudinal, and it seems probable that they assist in the rapid and considerable shortening of which this animal is capable when suitably stimulated. The muscles in the outer wall of the sinus of serpulids and sabellids are concerned with blood circulation.

![outline of capillary](image)

**FIG. 4.** Surface view of endothelium in living capillary of *Sabella spallanzanii*.

**The Endothelium**

The endothelium is visible in living coelomic capillaries of *Sabella spallanzanii* or *Branchiomma vesiculosum* as a reticulum of flattened cell bodies, connected with each other by strands of cytoplasm, and clinging to the inner surface of the skeletal coat (fig. 4). The nuclei are oval in surface view, are much flattened, and are arranged with their long axes parallel to the length of the capillary. The cytoplasm of both the cell-bodies and the strands contains numerous small yellow-brown globules, similar to the chloragosomes of the chloragogen cells. Staining with methylene blue makes the endothelium more conspicuous by tinting the nuclei pale blue and the globules dark blue.

When the living branchial vessel of *Sabella* is dissected out and examined in polarized light, the globules of the endothelium can be seen with careful focusing. The nuclei can then be identified as well-defined oval areas relatively free from globules, and resembling in shape and arrangement the nuclei of the capillary endothelium. The distribution of the globules in the endothelium of the branchial vessel suggests that it, too, is a reticulum.
In order to check the observation that the endothelium of *Sabella* is a reticulum and not a continuous layer, capillaries were treated by two methods for the silver impregnation of epithelial cell boundaries (Bergh, 1900, a, b; Romeis, 1932). The capillaries were first washed in a solution of potassium nitrate (Harmer, 1884) to prevent precipitation of silver chloride caused by the sea-water in which the worm had been dissected. No cell boundaries were visible in the endothelium or in any other layer of the capillary wall after treatment by either method, although the mesentery of a newt, used as a control in the experiment, was successfully impregnated.

The cell-bodies of the endothelium are conspicuous in sections of fixed capillaries and branchial vessels of *Sabella*, but the cytoplasmic strands connecting them are only occasionally detectable. It is not possible to demonstrate the reticular nature of the endothelium in these sectioned vessels, which can give only a very incomplete picture of the structure of this layer. Lack of observations on living vessels has no doubt been responsible for many of the controversies about annelid endothelia (see Hanson, 1949). In sections of *Sabella* stained with iron haematoxylin, small black globules are visible in the cytoplasm of the endothelium in all parts of the blood-system; they are presumably the chloragosome-like globules seen in living vessels. After careful differentiation of the stain, these black dots are clearly distinguishable from the grey granules of coagulated blood which sometimes cling to the endothelium.

The endothelium in the sectioned vessels of the following serpulids and a sabellid has the same appearance as the endothelium in sectioned vessels of *Sabella*, with the exception that no globules have been found in the cytoplasm: *Serpula, Hydroides, Vermiliopsis, Pomatoceros* (figs. 1, 7, and 9), *Salmacina, Protula, Apomatus, Spirorbis militaris, S. corrugatus*, and *Potamilla*.

The endothelium in all vessels in sections of the thorax of *Sabella* treated by the Prussian blue method was found to contain iron. The iron was presumably in some organic compound, because the reaction was negative unless the sections had been treated with acid before staining with potassium ferrocyanide. Masked iron is also present in large amounts in the chloragogen tissue and in the epidermis, particularly of the parapodia.

The presence of chloragosome-like globules and masked iron in the endothelium of *Sabella* gives rise to several suggestions concerning the functions of the endothelium. Chloragosomes have sometimes been regarded as accumulations of excretory substances, sometimes as food being transported round the body by the chloragogen-eleocyte system (Liebmann, 1942). Thus the globules in the endothelium might be waste substances or might be food being either extracted from or passed into the blood. The iron in chlorocruorin cannot be demonstrated by the Prussian blue method. A positive reaction from masked iron suggests that either a precursor or a breakdown product of the blood pigment may be present. It is possible that the endothelium plays a part in chlorocruorin metabolism. The chloragogen tissue of annelids, and
the heart body, an intravasal organ sometimes regarded as a kind of chloro-
gen tissue, have been shown to contain masked iron (Prussian blue method); and it has often been suggested that they are concerned in blood-pigment meta-
bolism (see Hanson, 1949). A heart body is absent in the Serpulimorpha.

Chloragosome-like inclusions were described by Vejdovsky (1905) in 'vaso-
thelial' (endothelial) cells of the dorsal vessel of the oligochaete Fridericia
hegemon. Nusbaum and Rakowsky (1897) saw similar inclusions in similar
cells of the dorsal vessels of F. ratzelli and F. striata; they considered that
these cells are not endothelial but represent a heart body.

Nusbaum (1905) noticed in regenerating blood-vessels of Amphiglena medi-
terranea a tissue composed of branched cells joined together by their processes. He thought that the tissue lay in the lumen of the vessel and was produced by the thin epithelium which constitutes the wall of the young vessel. He found that this tissue degenerates as the vessel grows. Nusbaum’s drawing of this tissue shows that it closely resembles the endothelium I have described in the coelomic capillaries of Sabella. A reticular endothelium of similar structure was found by Schneider (1908) in earthworms. Similar tissues in the walls of other annelid blood-vessels were described by others (see Hanson, 1949) but not interpreted as endothelia.

Lee (1912) held the opinion that an endothelium is absent in the vessels of serpulids (Serpula, Hydrodies, Vermiliopsis, Pomatoceros, Salmacina, Protula), and that here, as has often been suggested for other annelids (Hanson, 1949), blood corpuscles may temporarily adhere to the walls of the vessels and simulate an endothelium. Haswell (1885) admitted the presence of an endo-
thelium in Pomatoceros and Hydrodies, and suggested that it buds off blood corpuscles. I have never seen any cells free in the blood of the serpulids I have studied, which include all the genera used by Lee and Haswell. It is possible that in sections Lee and Haswell saw endothelial cells which sometimes adhere to the surface of the coagulated blood as it shrinks away from the wall of the vessel, but are easily recognized by their characteristic nuclei. Indeed, both Haswell and Lee recorded that the blood-cells are frequently situated near the periphery of the vessel and some are attached to its walls. Haswell, however, stated that he saw corpuscles moving in the blood in living Pomato-
ceros and Hydrodies, an observation which I cannot confirm.

The blood in both living and sectioned vessels of the following serpulids and sabellids has been found to lack blood-cells: Vermiliopsis, Pomatoceros, Salmacina, Spirorbis militaris, S. corrugatus, Sabella, Potamilla, Daisyhone. No blood-cells could be seen in sectioned vessels of Serpula, Hydrodies, and Protula or in living vessels of Amphiglena, Fabricia, Jasmineira, and Dialychone.

Blood-cells have been reported to be present in Sabella spallanzanii and Amphiglena mediterranea by Cuénot (1891), in Spirorbis borealis by zur Loye (1908), and in Sabella spallanzanii and S. pavonina by Romieu (1923). Ewer (1941), however, could not find them in the last two species. Apart from the possibility that endothelial cells adhering to the coagulated blood may sometimes have been mistaken for blood-cells, there are other sources of error.
which may account for previous reports that serpulids and sabellids have blood corpuscles. Endothelial cells occur on the fibrous bridles crossing the gut sinus, and without suitable staining methods the bridles may not be noticed. Lee (1912) apparently misinterpreted these endothelial cells as blood-cells in *Vermiliopsis* and *Protula*. Secondly, coagulated blood in sectioned vessels encloses small spherical cavities (fig. 2) which on casual inspection might be misinterpreted as blood-cells. Haswell's description of the blood-cells in sections of *Pomatoceros* and *Hydroides* suggests that he probably made this mistake.

The Skeletal Coat

The skeletal coat is a continuous sheet of a substance which gives the staining reactions of collagen, i.e. it is stained blue by Heidenhain's 'Azan' and red by van Gieson's picric acid/acid fuchsin, and it reacts negatively to the elastic fibre stains orcein and Weigert's resorcin-fuchsin. Although reticular fibres, impregnated with silver by Wilder's method, are present in the connective tissue of the rest of the body, I have not found any in the skeletal coat. Twerdochlebow (1917), however, found that the skeletal coat of members of the Aphroditidae can be impregnated with silver by Bielschowsky's method. In the Serpulimorpha, the skeletal coat is an apparently homogeneous membrane in which no fibres are visible. In polarized light the skeletal coat of living capillaries of *Sabella* is homogeneously birefringent. The birefringence is equally apparent however the capillary is oriented with respect to the plane of polarization of the illuminating rays. This suggests that the birefringent structures within the coat have no special orientation with respect to the long axis of the vessel. Thus I cannot confirm Hescheler's observation (in Lang, 1904) that the coat consists of longitudinal fibres. However, in contracted vessels of all serpulids and sabellids, the coat is longitudinally folded (fig. 1). Its thickness is apparently the same as in expanded vessels. Twerdochlebow (1917), however, has found that the skeletal coat in the dorsal vessels of *Aphrodite aculeata* and *Hermione hystrix* is considerably thicker in contracted than in expanded vessels.

Wherever the vessels are in contact with connective tissue, the skeletal coat in their walls is connected by fibres with the fibres in the connective tissue. For example, the skeletal coat in the outer wall of the gut sinus is continuous with the connective tissue fibres in the mesenteries and septa and also, by means of bridles crossing the sinus, with the basement membrane of the gut epithelium (figs. 2 and 3). The skeletal coat of the blood-system, like the basement membranes of the epithelia, is therefore to be looked upon as part of the connective tissue fibre system of the body, i.e. as part of the internal skeleton. The connexions between this coat and the rest of the skeleton no doubt serve to anchor the blood-vessels. The other functions of the coat are probably to resist any tendency to over-dilatation, and to distribute evenly the constricting effects of the contraction of the muscle-fibres, which do not form a continuous coat.
Narrow strands, consisting of the same material as the skeletal coat, extend across the gut sinus from the inner to the outer skeletal coats, and presumably prevent over-distension of the sinus. Endothelial cells, recognizable by their nuclei, are occasionally found on these fibrous strands. Such strands are present in *Serpula, Hydroides, Vermiliopsis, Pomatoceros, Salmacina, Protula, Apomatus, Spirorbis, Sabella* (fig. 3), *Potamilla*, and *Dasychone*. In the anterior part of the sinus of *Protula* and *Sabella* they are very numerous; they branch and anastomose with each other, and carry many endothelial cells. These strands were first described by Claparède (1873); he tentatively suggested that they might be contractile, and in places referred to them as 'brides contractiles'. However, they are apparently collagenous, they never contain muscle-fibres, and in a contracted sinus they are of approximately the same length as in an expanded sinus, but are folded. Lee (1912) found nucleated strands crossing the sinus of *Vermiliopsis* and *Protula*, and interpreted them as processes of blood-cells or as groups of blood-cells. Faulkner (1930) described 'protoplasmic strands' crossing the sinus of *Filograna implexa* from the gut epithelium to the peritoneum. Ewer (1941) showed that in *Sabella pavonina* and *S. spallanzanii* the bridles are formed of the same substance as the 'structureless layer', i.e. the skeletal coat, and bear scattered endothelial nuclei.

The Peritoneum

(a) General Histology

Outside the skeletal coat, in the walls of all parts of the blood-system of the sabellids and serpulids that have been examined in sufficient detail, there is a peritoneum containing muscle-fibres which lie in a single layer and are arranged approximately at right angles to the length of the vessel. The fibres are very narrow and difficult to see in small sectioned vessels, but they have been found in all vessels where they have been carefully sought (figs. 5 and 7). Thus there is evidence to support Claparède’s opinion (1873) that in the Serpulimorpha all vessels, even the smallest, have muscular walls.

The vessels fall into three categories, according to the structure of the peritoneum. These categories are: (i) The larger vessels, viz. the ventral, dorsal, and transverse vessels, the circum-oesophageal vessels, the branchial,
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 filament, and peduncle vessels, and the vessels of the outer peri-oesophageal plexus. (ii) The gut sinus. (iii) All the smallest vessels of the body, comprising all vessels not mentioned in the other two categories.

In contracted living coelomic capillaries of Sabella (category iii), examined unstained and in ordinary light, one can detect the muscle-fibres in profile view as grey-coloured dots, each situated above a slight constriction of the skeletal coat. In polarized light the fibres have a rather faint white birefringence, and can be seen in both the contracted and the expanded parts of the capillaries. Their arrangement is, however, more easily investigated in capillaries vitally stained with methylene blue (fig. 6).

Methylene blue was first employed for the vital staining of the muscles of annelid blood-vessels by Retzius (1891), and has since been used by several others (see Hanson, 1949). That the blue-staining fibres are indeed muscle-fibres is shown by the following observations. They are identical with the birefringent fibres seen when the capillaries are examined in polarized light. In contracted capillaries they constrict the skeletal coat and are wider than in expanded capillaries. In sections of capillaries, and in fixed preparations of whole capillaries, they can be identified with fibres which stain red with Azan. When a capillary is being stained with methylene blue, these fibres take up the stain at the same time as parapodial muscle-fibres.

When capillaries are immersed in an approximately 1 : 1,000 solution of methylene blue, prepared as described on p. 257, the muscle-fibres are stained a bright blue colour after about 15 minutes; under a coverslip they retain their colour for several hours before fading. It may be noted here that on no occasion have nerve-endings or nerve-fibres been seen on the walls of these capillaries, although Retzius (1905) and Federighi (1928) both noticed them during their studies on methylene-blue-stained vessels of Nereis. Carlson (1908) demonstrated nerve-cells on the vessels of Nereis and Arenicola by staining them with methylene blue, but found that the muscles failed to stain.

The blue-stained fibres on the capillaries of Sabella constitute a wide-meshed reticulum, in which the fibres all tend to lie transversely to the long axis of the capillary, so that the angles of forking are acute. One does not find any discrete fibres or any fibres with free ends. The nuclei of the peritoneum

![Fig. 6. Free-hand drawing of muscle-fibre reticulum, vitally stained with methylene blue, on surface of contracted coelomic capillary of Sabella spallanzanii. Outline of capillary out of focus.](image-url)
stain blue, and are sparsely scattered over the surface of the capillary. Their
distribution apparently bears no relation to the arrangement of the muscle-
fibres. These nuclei are few in number and difficult to detect. It is easier to
see them in capillaries which have been fixed and stained in a 1 per cent. solu-
tion of methyl green in 1 per cent. acetic acid, or in fixed preparations of whole
capillaries stained with iron haematoxylin. The nuclei appear oval in shape
when seen in surface view and, unlike endothelial nuclei, only slightly flattened
when seen from the side. Each has one nucleolus, which is sometimes very
conspicuous in methylene blue preparations, as well as in fixed preparations.
The scattered nuclei and the reticulum of muscle-fibres are embedded in an
apparently continuous sheet of cytoplasm constituting the outermost covering
of the capillary. The arrangement of the nuclei and fibres suggests that the
peritoneum is a syncytium. This suggestion is supported by the negative
results of attempts to demonstrate cell boundaries by silver impregnation.

Examination of fixed and sectioned capillaries of Sabella adds nothing new
to the picture of the peritoneum obtained by studies on the whole capillary.
One can see fine muscle-fibres and scattered nuclei lying in a coat of cytoplasm
covering the capillary; but it is not possible to see that the fibres are arranged
in a reticulum. The small vessels (category iii) of other sabellids and of serpu-
lids have been studied only in sections (fig. 5). Their peritoneum has the same
appearance as that of sectioned capillaries of Sabella, and it seems probable
that their muscle-fibres, also, are arranged in a reticulum.

The vessels in the pinnules, 'palps', thoracic membrane, and collar of
Pomatoceros differ from other small vessels in that the nuclei of the peritoneum
are arranged in a regularly-spaced single row along the length of the vessel.
In a pinnule, the vessel is attached to the basement membrane of the abfrontal
epithelium, and the nuclei belonging to its peritoneum lie in a row on the
mid-frontal face and project into the cavity of the pinnule (fig. 7). The same
holds true for the 'palps'. The vessels in the collar (fig. 8) and thoracic mem-
brane are accompanied by small channels situated between the two epithelia.
The vessel in each channel is attached to the basement membrane of the
ventral side, and its row of nuclei faces the dorsal side and projects into the
channel. The alignment of the nuclei in a single row may be due to restricted
space. But even on the filament vessels and the ventral vessel the nuclei show
a tendency to be arranged in rows along the length of the vessel (fig. 9). The
nuclei on the pinnule vessels of Serpula, Hydrodies, and Vermiliopsis are
arranged in the same manner as in Pomatoceros. A linear arrangement of the
nuclei of muscle-cells has been described in other annelids by Retzius (1891,
1905), Goodrich (1904), and Vejdovsky (1905) (see Hanson, 1949).

The peritoneum of the larger vessels (category i) and gut sinus (ii) differs
from that of the smaller vessels (iii) in that the muscle-fibres are independent
of each other and do not form a reticulum. Each fibre lies transversely to the
long axis of the vessel or sinus and extends only part of the way round it. The
fibres are wide and flat and interdigitate so as to form a single-layered muscle
coat, in which the gaps between the fibres are narrower than the fibres them-
FIG. 7. Longitudinal section through pinnule of Pomatoceros triqueter, to show structure of vessel wall.

FIG. 8. Section through collar of Pomatoceros triqueter, to show position of vessels.

Each fibre possesses one nucleus which lies on its outer surface (fig. 9). No other nuclei are present in the peritoneum. In sectioned vessels there appears to be cytoplasm in the gaps between the fibres. Thus the peritoneum of the larger vessels and the sinus, as compared with the peritoneum of the smaller vessels, is specialized in the differentiation of the reticulum of muscle-fibres into a coat of discrete muscle-fibres, and in the alinement of its nuclei, one to each fibre.
The peritoneum of the gut sinus differs from that of the larger vessels in two ways. Whereas the gut muscle-fibres are unbranched and rather narrow (fig. 10), those of the larger vessels are wider and may sometimes split into two; but the two branches lie close and approximately parallel to each other, and may join again. The branching of the fibres is clearly seen when one examines the isolated living branchial vessel of *Sabella* in polarized light. The second difference concerns the internal structure of the muscle-fibres (p. 269).

Like many annelid muscles (Prenant, 1929), those of the larger vessels and gut sinus in the Serpulimorpha are of the nematode type, i.e. the nucleus, surrounded by cytoplasm, is situated to one side of the fibre (figs. 9, 10, and 11). The nuclei are like those of the peritoneum of the smaller vessels—nearly spherical and with a single conspicuous nucleolus. Such nuclei seem to be characteristic of the muscles of annelid blood-vessels (Hanson, 1949). In the sabellids and serpulids I have examined, no other nuclei are found in the peritoneum. Thus the larger vessels and the sinus can be said to possess a muscle-epithelium. This conclusion agrees with that of Evenkamp (1931).

A muscle-fibre isolated from the sinus wall of *Sabella* by Goodrich's cell-dissociation method (Goodrich, 1942) shows the nucleus lying in a small membrane of cytoplasm which extends all the way along the fibre (fig. 10). Sometimes the membranes are slightly frilled, recalling the large frilled membranes of parapodial muscle-fibres of the Serpulimorpha and of the muscle-fibres of the hearts of *Lumbricus terrestris* (Hanson, 1948). The membrane can be identified in sections of the sinus, and also of the larger vessels, of both *Sabella* and *Pomatoceros* (fig. 12). A similar membrane was noticed by Bergh (1900b) on muscle-fibres of the larger vessels of *Lanice conchilega*. 

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**Fig. 9.** Longitudinal section through filament vessel of *Pomatoceros triqueter*. Surface view of wall.
(b) The Muscle-fibres

Some of the muscle-fibres of the blood-system of sabellids and serpulids have a complex internal structure, despite their small size. So far only preliminary observations have been made, most of them on sectioned vessels of *Pomatoceros* stained with iron haematoxylin.

![Diagram of muscle-fibres](image)

**Fig. 10.** Parts of muscle-fibres from gut wall of *Sabella spallanzanii*. (a) and (b) isolated by Goodrich's cell-dissociation method. (c) teased and treated with methyl-green-acetic.

![Diagram of muscle-fibres and vessel wall](image)

**Fig. 11.** Longitudinal section through wall of circum-oesophageal vessel of *Sabella spallanzanii*.

On the larger vessels each muscle-fibre apparently consists of a core of unstriped fibrils surrounded by a very thin sheath of cross-striated fibrils. Thus in longitudinal sections the fibre may appear to be striped or unstriped, or to be in part striped and in part unstriped, according to how it has been cut (fig. 12). In transverse sections only the core of unstriped fibrils has been
distinguished. Whole fibres (from the branchial vessels of *Sabella*) examined in polarized light appear to consist entirely of unstriped fibrils, presumably because the thin covering of cross-striated fibrils is masked by the totally birefringent unstriped fibrils lying underneath. The unstriped fibrils stain uniformly throughout their length and are uniformly birefringent. The cross-striated fibrils consist of alternating stained (anisotropic) and unstained (isotropic) zones of approximately equal length. Equivalent zones of adjacent fibrils lie side by side in bands across the muscle-fibre. Usually one can see only the alternating bands, but it is sometimes possible to distinguish the composite structure of each band, both stained and unstained.

The unstriped fibrils take an obliquely longitudinal course. Careful focusing sometimes reveals the existence of a second set of obliquely-longitudinal unstriped fibrils lying either above or below the first set. At one focal level the two sets of fibrils appear to cross each other, and the fibre possesses the so-
called ‘double-oblique’ striation frequently described in annelid muscle-fibres both of the blood-system (Hanson, 1949) and of the rest of the body (Prenant, 1929). It is particularly well shown by the muscle lamellae of the longitudinal body-wall musculature of the Serpulimorpha. A double-oblique striation has by some observers been attributed to a spiral arrangement of the fibrils, by others to the existence of two lamellae lying face to face and each possessing its own set of fibrils. In the case of the fibres of the larger vessels of serpulids and sabellids, I have no evidence to decide on either of these two alternatives.

These composite fibres therefore appear to consist of a core with a double-oblique striation ensheathed by fibrils with a true cross-striation. They have been found in sections of all the larger vessels of Pomatoceros, of the circumoesophageal and ventral vessels of Sabella, and of the filament vessels of Serpula, Vermiliopsis, and Protula.

The muscle-fibres of the gut sinus of the following serpulids and sabellids appear to consist entirely of unstriped fibrils: Serpula, Hydroides, Vermiliopsis, Pomatoceros, Apomatus, Protula, Sabella, and Potamilla.

The muscle-fibres of the smallest vessels of Pomatoceros and Sabella are often apparently homogeneous in structure, but sometimes show alternating stained (anisotropic) and unstained (isotropic) zones. The fibres of living capillaries of Sabella show no striation even in polarized light.

Muscles, other than those of the blood-system, in all the serpulids and sabellids examined consist only of unstriped fibrils.

**DISCUSSION**

The observations reported in this paper have already been reviewed (Hanson, 1949) together with the extensive literature on the histology of the blood-system in the Oligochaeta and Polychaeta. In this discussion I shall briefly compare the Serpulimorpha with other annelids.

The walls of the blood-system in all oligochaetes and polychaetes typically consist of three layers, a skeletal coat covered on the outer side by a peritoneum, and on the inner side by an endothelium.

Numerous observations on the endothelium have shown that the cells and their nuclei are much flattened, and that the nuclei are elongated parallel to the length of the vessels. Additional information is scanty. Schneider (1908) has given a fuller description of the endothelium of Lumbricus, agreeing with my observations on Sabella. In both cases the endothelium is a reticulum of branched cells joined together by their processes. L. Dehorne (1916—naids), Vejdovsky (1905—Potamotheix, enchytraeids, Rhynchelmis, Dendrobaena), and Twerdochlebow (1917—Aphroditidae) have all described an endothelium consisting of discrete bipolar cells elongated parallel to the length of the vessel and containing fibrils asserted but not demonstrated to be contractile. Sterling (1909) and A. Dehorne (1936) could not confirm the observations of Vejdovsky and Twerdochlebow.
The skeletal coat of all oligochaetes and polychaetes consists of a homogeneous collagenous membrane which becomes longitudinally folded when the vessel contracts.

The peritoneum varies in structure. In a few cases (lumbricids—Gungl, 1905; *Nereis*—Federighi, 1928), as in serpulids and sabellids, it has been shown that the muscle-fibres become better differentiated as one passes from the smaller to the larger vessels. According to Gungl (1905) and Schneider (1908) the peritoneum of lumbricid capillaries consists of a flat epithelium containing intracellular fibrils; apparently these capillaries resemble those of serpulids and sabellids. Plenk (1925), however, has described stellate muscle-cells, like the Rouget cells of vertebrate capillaries (Krogh, 1929), on lumbricid capillaries. Similar cells have been found on the smaller vessels of several oligochaetes and polychaetes (e.g. L. Dehorne, 1916; Retzius, 1891, 1905). In vertebrates, transitional forms between the Rouget cells of the capillaries and the smooth muscle-cells of the larger vessels have been described. A similar transition from stellate muscle-cells to muscle-fibres with few or no branches was found in *Nereis* by Retzius (1905) and Federighi (1928).

On the larger vessels of oligochaetes and polychaetes the muscle-fibres are arranged in one or more muscle coats which may be covered by a peritoneal epithelial layer (e.g. Aphroditidae—Twerdochlebow, 1917). In many cases, however, as in serpulids and sabellids, the peritoneal epithelium is absent or incomplete, and the cell-bodies of the muscle-fibres project on the surface of the vessel. The peritoneum is thus a muscle-epithelium.

There have been numerous accounts of the structure of the muscle-fibres (e.g. Vejdovsky, 1905). They all agree that, as in serpulids and sabellids, the fibres are of the nematode type, i.e. consist of a cell-body containing the nucleus and processes containing the myofibrils. The nucleus is nearly spherical and has a single nucleolus.

Various descriptions have been published of the internal structure of the muscle-fibres. In some cases (e.g. lumbricids—Gungl, 1905; Aphroditidae—Twerdochlebow, 1917) the myofibrils are said to be unstriated, but so arranged that the fibre has a double-oblique striation. On the contrary, Plenk (1925) concluded that the muscle-fibres on the vessels of lumbricids show a genuine cross-striation of the myofibrils. Goodrich (1942), however, found neither cross-striation nor double-oblique striation of fibres from the hearts of *Lumbricus*. The muscle-fibres of the blood-systems of serpulids and sabellids appear so far to be unique in possessing both striped and unstriped myofibrils.

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