The connective tissue sheath of the locust nervous system: its development in the embryo

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With 4 plates (figs. 1 to 4)

Summary

The embryological development of the connective tissue sheath around the nervous system has been investigated in *Schistocerca gregaria*. The sheath cells appear to be derived from outlying ganglion cells. The neural lamella is first visible when the embryo is 9 days old and it increases in thickness until hatching occurs on the twelfth day. It is produced entirely by the sheath cells.

The sheath cells have numerous lipid droplets in their cytoplasm. Some neutral mucopolysaccharide and proteins are also present. The histochemical reactions of the neural lamella after its formation suggest that it is composed of collagenous proteins embedded in neutral mucopolysaccharides.

The sheath cells are typical fibroblasts during the formation of the neural lamella. The cisternae of the endoplasmic reticulum are dilated into vesicles which contain a somewhat electron-dense material. No intracellular fibrils were observed. Collagen fibrils with banding of periodicity between 55 and 60 μ are seen in the neural lamella from 11 days onwards.

Introduction

It has been known for some time that the connective tissue sheath, which surrounds the nervous system of insects, consists of an outer layer, the neural lamella, overlying a layer of sheath cells, and that the neural lamella is composed of collagen fibrils embedded in mucopolysaccharides (Ashhurst, 1959, 1961, 1964; Ashhurst and Richards, 1964a, b; Baccetti, 1955, 1956; Hess, 1958; Smith and Wigglesworth, 1959). The production of insect connective tissues was attributed to the haemocytes by Lazarenko (1925), Wermel (1938a, b), and Wigglesworth (1956), though the latter author considered that the underlying cells might have some part in their formation; Scharrer (1939) suggested that the neural lamella was produced solely by the cells beneath. In order to investigate fully the process of fibrillogenesis in insects, morphological, histochemical, and electron-microscopical studies were made of the developing adult connective tissue sheath of the abdominal region of the nerve cord in the pupa of the wax moth, *Galleria mellonella*; the results obtained indicated that the sheath cells are responsible for the production of the fibrous tissues, and that the developing tissue has several features in common with developing vertebrate connective tissues (Ashhurst, 1964; Ashhurst and Richards, 1964a, b).

The electron-microscopical study of the moth tissue showed that the fibrils present are not typical collagen fibrils with banding of 640 Å periodicity (Ashhurst, 1964), although the histochemical reactions of the tissue and

the presence of hydroxyproline in hydrolysates of the tissue indicated that collagenous proteins are present (Ashhurst and Richards, 1964b). Thus, while there is now information about the production of the atypical collagen of the Lepidoptera during metamorphosis, nothing has been known about the production of typical collagen fibrils, or about the development of connective tissue in an insect embryo.

Embryological studies of insects invariably end as soon as organogenesis is complete, and although the connective tissue cells are differentiated by this time, the layers of fibrous connective tissue have not yet been produced. There is some controversy about the origin of the sheath cells of the nervous system, which will be discussed later in detail, as some workers think that they are derived from outlying ganglion cells, while others consider that they are of mesodermal origin (Johanssen and Butt, 1941).

The embryonic development of the connective tissue sheath in the locust, *Schistocerca gregaria*, was studied by both light and electron microscopy. Since the collagen fibrils of locusts (*Locusta migratoria* and *Schistocerca gregaria*) are similar to vertebrate fibrils (Ashhurst and Chapman, 1961; Melis, 1961), this investigation provided information about the production of 'typical' collagen fibrils, in addition to that about the early development of the connective tissue sheath. The results of this research are described in this paper.

**Materials and methods**

The eggs of *Schistocerca gregaria* used in this work were provided by the Anti-Locust Research Centre, London, S.W. 7. This species was chosen because the eggs could be supplied in large numbers and, when kept at 32°C, hatching occurs on the twelfth day.

Before fixation, the embryos were dissected from the egg cases and as much yolk as possible was removed from the younger embryos. For the morphological study, embryos from 3 to 11 days old and newly hatched nymphs were fixed in either Bouin's fluid at 60°C or in Zenker's fluid at room temperature overnight, and then embedded in wax. Serial sections were cut and those from embryos from 3 to 5 days old were stained in Heidenhain's iron haematoxylin, while the later stages were stained with Masson's trichrome stain.

The embryos to be used for histochemical study were fixed in either formaldehyde-saline overnight, formaldehyde with 1% cetylpyridinium chloride for 48 h (Williams and Jackson, 1956), formaldehyde-calcium followed by postchromation (Baker, 1946), or Zenker's fluid overnight. The material for lipid tests was embedded in gelatin, the rest in wax. The histochemical tests performed were as follows:

**Carbohydrates.** The periodic acid / Schiff (PAS) reaction with diastase controls (Pearse, 1960) was done on sections fixed in formaldehyde-saline, formaldehyde with cetylpyridinium chloride, and Zenker's fluid. Various tests for acid mucopolysaccharides were performed on sections fixed in formaldehyde with cetylpyridinium chloride; these included the alcian blue test.
Ashhurst—Connective tissue of locust nervous system

(Pearse, 1960), and both azure A and toluidine blue for metachromasia (Baker, unpublished; Casselman, 1959).

**Lipids.** Frozen sections of the tissue fixed in formaldehyde-calcium were coloured with Sudan black B (Baker, 1956b) and other sections were taken through the acid haematein test for phospholipids (Baker, 1946). Some embryos were subjected to the pyridine extraction test (Baker, 1946).

**Proteins.** Sections fixed in formaldehyde-saline were tested for the presence of arginine with the Sakaguchi reaction (Baker, 1947), for tryptophane with the p-dimethylaminobenzaldehyde (DMAB) reaction (Pearse, 1960), and for tyrosine with the Hg / nitrite test (Baker, 1956a).

For examination with the electron microscope, embryos from 9 days onwards and newly hatched and 2-day nymphs were dissected quickly; the gut was removed, and the embryo then flooded with the osmium fixative. The fixative was 1% osmium tetroxide in veronal-acetate buffer at pH 7.4, with trace amounts of calcium and magnesium chlorides added. The tissue was dehydrated in graded ethanols and passed through propylene oxide to araldite for embedding. Thin sections of the central nervous system were cut on a Huxley ultramicrotome. The sections were stained with either lead citrate (Reynolds, 1963), 1% phosphotungstic acid in absolute ethanol, 2% uranyl acetate in methanol, or vanadatomolydbate (Callahan and Horner, 1964). They were examined in a Siemens Elmiskop I electron microscope.

**Results**

*Embryological development of the connective tissue sheath*

The developing nervous system is easily distinguished in an embryo 3 days old. The neuroblast cells lie on top of the hypodermis and the newly formed neurones can be seen in each segment. No neuropile is yet formed. At 4 days, while the nervous system has not yet separated from the hypodermis, a small amount of neuropile occurs in each ganglion. It appears from serial sections that the masses of cells forming the ganglia have now, towards their dorsal side, separated to allow the neuropile to develop in the space between the neurones (fig. 1, A, B). In the central area of the dorsal side of the ganglia, the layer of cells over the neuropile is very thin, and probably consists of the sheath cells, or in the terminology adopted in embryological studies, the 'neurilemma' cells. The thin layer of cells is not very clear, but at this stage it appears to cover only a small part of the dorsal surface of each ganglion. By 5 days, the nervous system of the embryo has separated from the hypodermis and with the elongation of the embryo some of the ganglia move apart, leaving connectives between them. The neuropile has increased in size and the whole nervous system is now surrounded by a thin layer of cells with flattened nuclei (fig. 1, C, D); that is, the sheath cells now form a complete layer.

It is extremely difficult to trace the origin of the sheath cells with any certainty. There are two obvious alternatives: the cells are either derived from
outlying ganglion cells, or from mesodermal cells that have spread over the nervous system. In the ganglia of a 3-day embryo, all the cells of the nervous system appear of similar shape and size, but on the dorsal side of the 4-day embryo, a layer of flattened cells is present over a small area (fig. 1, B). These cells have no obvious connexion to any cells of the embryo outside the nervous system and it is suggested that in *Schistocerca* the sheath cells are formed from outlying ganglion cells, as was suggested for the sheath cells of *Locusta* by Roonwal (1937).

The nervous system and its sheath cells continue to develop (fig. 1, E), but the neural lamella cannot be clearly distinguished until the embryo is 9 days old. The embryo now completely fills the egg case and externally it appears to be fully developed. Internally the nervous system is seen as a series of 10 ganglia with connectives between. During development there is fusion of the third thoracic and first 3 abdominal ganglia and of the last 4 abdominal ganglia, so that the nervous system before hatching is composed of a cerebral, suboesophageal, 3 thoracic, and 5 abdominal ganglia. The neural lamella is not found until the nervous system has reached this stage of development. This corresponds to the situation in the pupae of *Galleria mellonella*, where the neural lamella of the adult is not formed until the larval nervous system has completed its transformation to the adult form.

In the 9-day embryo, the neural lamella can be seen in Masson preparations as a green layer about 0.5 μ thick surrounding the entire nervous system. The sheath cells beneath it are a layer of cells varying in width from 1 to 3 μ. The nuclei of these cells are elongated; they are between 9 and 13 μ in length, but only 2.5 to 3.5 μ in width. The neural lamella and sheath cells of an embryo 10 days old are seen in fig. 1, F. By 11 days the neural lamella has increased in width to about 1 μ, and the sheath cell layer may be as much as 5 μ in thickness in some places. The connective tissue sheath is in this form when the nymph hatches (fig. 1, G).

The neural lamella appears to be produced solely by the sheath cells which underlie it. At no time during its development were blood cells present around the nervous system. The only cells which appear anywhere in the vicinity

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*Fig. 1* (plate) is a series of photomicrographs. A, B, C, and D are stained with iron haematoxylin; E, F, and G are stained with Masson's trichrome stain.

A, a transverse section through a ganglion of an embryo 4 days old. A small area of neuropile (np) can be seen towards the dorsal region, separating the neurones (n).

B, a transverse section through the centre of a ganglion of a 4-day embryo about 10 μ away from the section shown in fig. 1, A. The area of neuropile (np) is larger and a layer of cells (sc) is more apparent over the dorsal surface.

C, a longitudinal section through a ganglion of an embryo 5 days old. The neuropile (np) can be seen amongst the neurones (n) with a single layer of cells (sc) on its dorsal side.

D, a longitudinal section through the same ganglion as in fig. 1, C, but through the centre. The layer of sheath cells (sc) over the neuropile (np) is clearly differentiated.

E, the sheath cells (sc) of an embryo 6 days old overlying several neurones (n). The nuclei (nu) of the sheath cells are flattened.

F, the sheath cells (sc) and the newly formed neural lamella (nl) in an embryo 10 days old.

G, the sheath cells (sc) and neural lamella (nl) of a newly hatched nymph.
are the fat-body cells. In the abdominal region the nervous system is surrounded by the developing fat-body, and while in scattered places they appear to be in very close contact, these areas are so restricted in number and size that it is considered that the fat-body has no direct role in the production of the neural lamella.

**Histochemical observations**

The histochemical reactions of the neural lamella and sheath cells were observed in embryos ranging from 7 to 11 days and in newly hatched nymphs. The range covers the period in which the neural lamella is produced. There are no changes in the reactions of the sheath cells during this period, nor in those of the neural lamella after its appearance at 9 days. Hence in the succeeding discussion of the histochemistry of the neural lamella and sheath cells, no reference will be made to the stages of development. The results of the histochemical tests are summarized in table 1.

**Table 1**

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<th>Test</th>
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<th>Sheath cells</th>
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<td>PAS with no oxidation</td>
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<td>pyridine extraction</td>
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<td>Sakaguchi</td>
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Key: ++ + = strong reaction, ++ = moderate reaction, + = weak reaction, o = no reaction.

**Sheath cells.** The reaction of the cytoplasm of the sheath cells to the PAS test is weakly positive and it is not affected by diastase digestion. This suggests that while the cytoplasm contains some neutral mucopolysaccharide, no glycogen is present. After coloration with Sudan black B, numerous lipid droplets can be seen in the cytoplasm of the sheath cells. The droplets also give a positive reaction with the acid haematein test, which suggests that they contain some phospholipids. No attempt was made to characterize the lipid further. All three protein tests were positive in the cytoplasm.
The histochemical reactions of the sheath cells of the embryo are similar to those of the adult locust, except for the apparent absence of glycogen in the cells; this will be discussed more fully later.

**Neural lamella.** From its formation onwards the neural lamella has the same histochemical reactions as the neural lamella of *Locusta migratoria* (Ashhurst, 1959). It gives a positive reaction with the PAS test, and this is not affected by diastase digestion; this indicates the presence of neutral mucopolysaccharides. For acid mucopolysaccharides, the alcian blue test and both the metachromatic dyes, azure A and toluidine blue, were tried; but in all the experiments a negative result was obtained. Thus it must be concluded that acid mucopolysaccharides are not present in histochemically detectable quantities in the developing neural lamella.

The neural lamella is not coloured by Sudan black B, nor does it give a positive reaction with the acid haematein test. Therefore, histochemically detectable lipids are not present.

The Sakaguchi and Hg / nitrite tests both produced positive reactions in the neural lamella, but the result with the DMAB test was negative. Thus while arginine and tyrosine are present in the neural lamella, tryptophane does not occur in detectable quantities.

It is known that collagen is present in the neural lamellae of *Locusta* and *Schistocerca* adults (Ashhurst, 1959; Ashhurst and Chapman, 1961; Melis, 1961) and as the histochemical reactions of the embryo neural lamella are the same as those of the adult locust neural lamella, it seems reasonable to assume that some collagen production occurs in the embryo. Two points must, however, be mentioned. Acid mucopolysaccharide is usually associated with developing connective tissue, and none is present in this developing tissue, and while in the adult locust the reaction to the Hg / nitrite test was weaker than that to the Sakaguchi test, in the embryo they are more or less equal in strength. This discrepancy may be due in part to the fact that the protein tests rarely give very strongly coloured reaction-products, and the neural lamella is a very thin membrane at this stage.

**Electron microscopy**

Most of the observations were made on embryos of 9 to 11 days, and on newly hatched and 2-day nymphs.

**Fig. 2 (plate).** A, a low-magnification electron micrograph of the sheath in an embryo 9 days old. The neural lamella (nl) is seen as a thin amorphous layer on the surface of the sheath cells (sc). Part of a nucleus (nu) is visible. The cytoplasm contains a Golgi zone (g), a few mitochondria (m), some vesicles (v) of the endoplasmic reticulum, and free ribosomes (r). The plasma membrane (pm) is discontinuous on the outer surface of the cells (arrows).

B, a low-magnification electron micrograph of the sheath in an embryo 10 days old. The endoplasmic reticulum (the sheath cells (sc) is now in the form of cisternae (c) with ribosomes and vesicles (v). The neural lamella (nl) is still amorphous and does not form a distinct layer.

C, part of the neural lamella of an embryo 11 days old. A distinctly banded collagen fibril (c) is seen amongst the amorphous material. Many other fibrils (f) with less distinct banding are present.
FIG. 2
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FIG. 3

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**Sheath cells.** The sheath cells form a layer in the 9-day embryo between 1 and 3 μ thick and this increases in width so that it may be 5 μ in places in the 11-day embryo and newly hatched nymph. During the 9 to 11 day period, when the neural lamella is being produced, the cytoplasm of the sheath cells becomes more closely packed with cytoplasmic inclusions. The sheath cells of a 9-day embryo are seen in fig. 2, A; the cytoplasm is sparsely filled. The endoplasmic reticulum is seen as a few small vesicles with some ribosomes. By 10 days the endoplasmic reticulum is much better developed and consists of cisternae, some of which are dilated at the ends, and some vesicles with contents hardly more electron-dense than the surrounding cytoplasm (fig. 2, B). The endoplasmic reticulum continues to develop and the number of vesicles increases. These appear to contain a more electron-dense material in the 11-day embryo (fig. 3, A), and their contents may be tropocollagen molecules, together with some of the mucopolysaccharides usually associated with collagen. Throughout the 9 to 11 day period the outer plasma membrane of the sheath cells is irregular and in some places it seems to be absent; the plane of the sections and the nature of these interruptions in the cell membrane suggest that they are real and not artifacts. In some places in the 11-day embryo, the plasma membrane has large indentations, the contents of which are similar to the contents of the vesicles of the endoplasmic reticulum (fig. 3, C). It appears possible that these might be vesicles which have come to the surface of the cells to discharge their contents into the developing neural lamella, in a manner which might be described as merocrine secretion; this is, however, only a suggestion since there is no direct evidence for such a mechanism. In the nymphs, the endoplasmic reticulum is not so prominent as in the 11-day embryo. It consists of small cisternae with ribosomes. The outer plasma membrane is intact, separating the cells from the neural lamella (fig. 4, A, B). In all stages, free ribosomes are observed in the cytoplasm.

During this developmental period the mitochondria increase in number, so that in the nymphs the sheath cells are packed with them. Lipid droplets with irregular outlines are seen in many sections (fig. 3, B). Small Golgi areas are present in some sections, but they are not well developed (fig. 2, A; fig. 3, A). The membranes separating the sheath cells are clearly visible, especially in the nymphs; it appears in some areas that the sheath cells interdigitate with each other, since so many membranes can be seen in one section (fig. 4, B).

**Neural lamella.** In the 9-day embryo the neural lamella, though it appears as a distinct layer with the light microscope, appears as an irregularly formed,
loosely packed layer of amorphous material (fig. 2, A); no fibrils can be seen. It is not until the embryo is 11 days old that the neural lamella is a clearly defined layer, and at this stage, banded fibrils may be seen among the amorphous material in a few areas (fig. 2, c). The number of visible collagen fibrils increases greatly by the time the embryo is 2 days old (fig. 4, A, B, C).

The amorphous material is probably a mixture of mucopolysaccharides and unpolymerized collagen molecules. Intracellular fibrils were not observed in *Schistocerca*, and it is thought that the formation of the collagen fibrils from the tropocollagen molecules produced in the endoplasmic reticulum is entirely extracellular. Fibrils are not observed until the 11th day. Before this, polymerization has possibly not proceeded far enough for fibrils to be visible; the density of the amorphous material would make fibrils of small diameter difficult to distinguish. The periodicity of the banding of the collagen fibrils is between 55 and 60 μm; 6 interbands per period are clearly visible (fig. 4, c, d). This is similar to the banding of the fibrils in the neural lamellae of adult *Locusta migratoria* and *Schistocerca gregaria* (Ashhurst and Chapman, 1961; Smith and Treherne, 1963). The fibrils are rather small; their diameter is between 15 and 40 μm. The neural lamella is not clearly differentiated into layers, except that there is a layer of amorphous material, similar to that forming the neural lamella of the 9-day embryo, on its outer surface (fig. 4, c). There is no organized pattern of orientation among the collagen fibrils.

**Discussion**

While this paper is primarily concerned with the formation of the collagen fibrils of the neural lamella by the sheath cells, some consideration of the origin of these cells is necessary. In *Schistocerca*, it seems that the sheath cell layer, or 'outer neurilemma' as it is usually called in embryological studies, is derived from the outer ganglion cells at approximately the time when the nervous system is separating from the hypodermis. This is also thought to be their origin in *Apis mellifica*, *Corynodes pusis*, *Eutermes*, *Hierodula*, *Locusta migratoria*, *Melanoplus differentialis*, *Pieris rapae*, and *Xiphidium eusiferum* (Baden, 1936; Eastham, 1930; Görg, 1959; Nelson, 1915; Paterson, 1935; Roonwal, 1937; Strindberg, 1913; Wheeler, 1893). Heymons (1895) considered that the neurilemma cells in *Forficula* were derived from the dermagenous layer when the nervous system separated from it; Patten (1888)

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Fig. 4 (plate). A, a low-magnification electron micrograph of the sheath of a nymph 2 days old. The neural lamella (nl) contains many fibrils. The cytoplasm of the sheath cells (sc) is now more compact. Mitochondria (m), a few small vesicles (v) of the endoplasmic reticulum, and some ribosomes (r) are present.

B, part of the sheath of a 2-day nymph to show the large number of plasma membranes (pm) seen in some areas.

C, part of the neural lamella of a 2-day nymph at higher magnification. Collagen fibrils (c) are present in both longitudinal and transverse section. A thin layer of amorphous material (a) is present on the outer surface.

D, a collagen fibril (c) from the neural lamella of a 2-day nymph which shows the six interbands in each major banding period.
thought that they were formed in a similar way in *Acilius*. Another suggestion about their origin was made by Tiegs and Murray (1938), who thought that in *Calandra oryzae* the sheath cells of the brain were derived from outlying ganglion cells, and those of the ventral nerve cord from columnar cells associated with the median cord cells in the intersegmental portions of the nervous system. All these derivations assume that the sheath cells are of ectodermal origin, but Nusbaum (1886) suggested that they originate from yolk cells in *Blattella germanica*, while Korotneff (1885) suggested that they are produced by amoeboid mesodermal cells in *Gryllotalpa*. The question of the origin of these cells has still to be settled, but the difficulties encountered in determining the derivation of a single layer of cells is perhaps reflected in the fact that the ‘neurilemma’ is not even mentioned in the majority of the more recent embryological studies of insects.

In the present investigation blood cells were never observed in association with the nervous system in the embryo or young nymph, and it is therefore concluded that they play no part in the production of the neural lamella. The sheath cells appear to be responsible for the production of both the collagenous protein and the mucopolysaccharides. This is essentially similar to the situation in the pupa of *Galleria mellonella* (Ashhurst, 1964; Ashhurst and Richards, 1964a, b), where the adult neural lamella and dorsal mass of connective tissue are the product of the sheath cells. Participation in connective tissue formation by haemocytes has been suggested by several authors (Lazarenko, 1925; Wermel, 1938a, b; Whitten, 1964; Wigglesworth, 1956), but the suggestion made by Scharrer (1939) that the neural lamella of *Periplaneta americana* is secreted by the underlying cells is in agreement with the observations presented in this paper. Occasionally the fat-body cells are in close association with the sheath cells. The infrequency of this association makes it improbable that they play a major role in the production of the neural lamella, but it is possible that they aid the nutrition of the nervous system in a similar way to that suggested by Smith and Treherne (1963), who found an extremely close association between the fat-body and central nervous system in *Periplaneta*.

The histochemical reactions of the sheath cells indicate that they are very similar to the sheath cells of the adult *Locusta migratoria* and *Periplaneta americana* (Ashhurst, 1959, 1961) in the possession of lipid droplets and mucopolysaccharides in the cytoplasm. The absence of glycogen is noteworthy, especially as Wigglesworth (1960) considers it to be very important in the nutrition of the central nervous system; it has been found in abundance in the sheath cells of *Galleria mellonella, Locusta migratoria*, and *Periplaneta americana* (Ashhurst, 1959, 1961; Ashhurst and Richards, 1964b; Wigglesworth, 1960). The newly hatched nymph is very active despite the lack of glycogen; no histochemical observations were made on older nymphs, but it is possible that these do have glycogen in the sheath cells.

The main features of the sheath cells revealed by electron microscopy suggest several basic similarities with vertebrate fibroblasts. The endoplasmic
reticulum of fibroblasts is typically dilated and the resulting dilations and vesicles contain substances that are more electron-dense than the surrounding cytoplasm. This form of endoplasmic reticulum is present in the fibroblasts of *Galleria* and many vertebrates (Ashhurst, 1964).

The Golgi areas are very poorly developed in the sheath cells of *Schistocerca* and it would appear that they do not play a very active role in fibrillogenesis in this species. In *Galleria*, no Golgi bodies were observed (Ashhurst, in press). Using autoradiographic techniques, Revel and Hay (1963) found that in chondrocytes of *Amblystoma* the products of the endoplasmic reticulum are transferred to the Golgi vesicles before they are extruded from the cell, in a manner analogous to the stages of secretion in exocrine gland cells. It was mentioned in a previous paper (Ashhurst, 1964) that Golgi areas may be more highly developed in chondrocytes than in other fibroblasts.

Vertebrate fibroblasts can be divided into 2 groups according to whether or not they possess fibrils in their cytoplasm (see Ashhurst, 1964). These are commonly considered to represent a stage in the polymerization of the tropocollagen molecules, but Goldberg and Green (1964) present evidence, based on amino-acid analyses, that the intracellular fibrils in cultured mouse fibroblasts are not collagenous. A similar distinction may be found in insect fibroblasts, since intracellular fibrils are found in the fibroblasts of *Galleria* (Ashhurst, 1964), but not in those of *Schistocerca*. In the latter species, it seems that the tropocollagen molecules are passed out of the cells before the fibrils are formed. It has been suggested that the cell membrane is important in providing a template for the polymerization of the collagen molecules (Porter and Pappas, 1959) and it may perform this function in *Schistocerca*.

The discontinuities in the outer plasma membrane of the sheath cells of *Schistocerca* have been mentioned. These are quite small and limited in area compared to the areas lacking a plasma membrane in the sheath cells forming the dorsal mass in *Galleria*. The plasma membrane of vertebrate fibroblasts may appear to be discontinuous (Chapman, 1961; Peach and others, 1961; Yardley and others, 1960), but Goldberg and Green (1964) consider that such breaks in the plasma membrane are not real, but due to the angle of the plasma membrane in relation to the plane of the section. In *Schistocerca* the breaks in the plasma membrane are abrupt, and the plane of the sections is perpendicular to the sheath cell layer; so it is suggested that they are real and not artifacts. They may be concerned with the passage of substances from the cells to the neural lamella.

In the 11-day embryo, when collagen secretion to the neural lamella is very active, indentations of the plasma membrane are found. It appears as if vesicles of the endoplasmic reticulum may approach the plasma membrane, join with it, and then extrude their contents into the neural lamella. Evidence for a similar method of extrusion of the contents of the endoplasmic reticulum and Golgi vesicles has been found in vertebrate fibroblasts and chondrocytes by Godman and Porter (1960), Goldberg and Green (1964), Karrer (1960), and Revel and Hay (1963).
The absence of acid mucopolysaccharides in histochemically detectable amounts in the developing neural lamella of *Schistocerca* is in sharp contrast to the abundance of these substances in *Galleria* (Ashhurst and Richards, 1964b). Acid mucopolysaccharides are usually abundant at sites of collagen production in vertebrates, although it has been pointed out that they may not be essential in high concentration for fibril formation, since the amount of acid mucopolysaccharide in healing wounds is less than that in the surrounding skin (Jackson and others, 1960). In experiments on the precipitation of collagen from solutions *in vitro*, fibrils with 640 Å banding can be produced in the absence of acid mucopolysaccharides (Keech, 1961). In a previous paper (Ashhurst, 1964) it was suggested that the high concentration of acid mucopolysaccharide in the developing connective tissue of *Galleria* may in some way contribute to the small diameter and indistinct banding of the fibrils, since Wood (1961) found that large amounts of acid mucopolysaccharide caused the production of fibrils of small diameter *in vitro*. In contrast, the fibrils in *Schistocerca*, produced in the absence of large amounts of acid mucopolysaccharide, are more highly organized in their banding and are of greater diameter. There is no direct evidence, however, that the difference in the fibrils of the two species can be correlated with the amounts of acid mucopolysaccharide present. Typical collagen fibrils are found in the neural lamellae of other orders of insects (Baccetti, 1961), but, apart from the cockroach, in which acid mucopolysaccharides are not present (Ashhurst, 1961), their mucopolysaccharide content is not known.

If, as is suggested in this paper, the sheath cells are derived from ganglion cells, they are ectodermal in origin. Collagen production is usually associated with cells of mesodermal origin, but some other collagens, for example the cuticles of earthworms and nematodes, are secreted by ectodermal epithelia (Gross and Piez, 1960). Hay and Revel (1963) suggest that the epidermal cells of *Amblystoma* may secrete both collagen and keratin.

The results described in this paper present further evidence for the fundamental similarities between fibroblasts in the vertebrates and insects. The fact that the sheath cells are probably concerned with other functional aspects of the nervous system does not detract from this, since some vertebrate cells capable of producing collagen serve other functions (see Ashhurst, 1964). It is perhaps not surprising to find that an important structural protein is produced in a similar manner in widely differing animals.

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References

PATTEN, W., 1888. J. Morph., 2, 97.
Ashhurst—Connective tissue of locust nervous system

WHEELER, W. M., 1893. J. Morph., 8, 1.