Fibrillogenesis in the wax-moth, *Galleria mellonella*

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With 6 plates (figs. 1 to 6)

**Summary**

The fibroblasts of the pupa are characterized by the great development of the endoplasmic reticulum, which becomes dilated to form vesicles containing a rather electron-dense material which is thought to be a precursor of the collagen fibrils. Fibrils are seen within the cytoplasm of the fibroblasts; these are about 12.5 to 20 μ in diameter and some of them show indications of banding with a periodicity between 15 and 20 μ. It is thought that these fibrils and their surrounding cytoplasm become incorporated into the fibrous connective tissue. The plasma membranes of the fibroblasts are discontinuous where they are adjacent to the fibrous tissue. The fibrils in the connective tissue are obscured by masses of mucopolysaccharide, but there are indications that these fibrils are essentially similar to the intracellular fibrils.

The processes of fibrillogenesis in the moth and in various vertebrate tissues are shown to have many features in common.

**Introduction**

In the past decade, a vast amount of information has been accumulated about the formation of connective tissues in the vertebrates, but, although collagenous connective tissue is known to be present in several invertebrate phyla, its formation has attracted little interest. The nature of the collagen present in the cuticle of earthworms and *Ascaris*, in the byssus threads and body wall of molluscs, in the Cuverian tubules of Holothuria, and in the body walls of coelenterates and sponges has been well characterized (Bird, 1957; Gross and Piez, 1960; Jackson and others, 1953; Maser and Rice, 1962; Melnick, 1958; Watson, 1958; Watson and Silvester, 1959), but the only study of fibrogenesis has been made on the leech, *Hirudo medicinalis* (Bradbury and Meek, 1958), where the collagen fibrils were observed 'shredding off' from the plasma membrane of the fibrocyte.

In the insects, connective tissue is restricted to thin sheets around the various organs. Histochemical studies have indicated that the connective tissue is composed of collagenous proteins embedded in a matrix of mucopolysaccharides (Ashhurst, 1959, 1961; Ashhurst and Richards, 1964a, b; Baccetti, 1955, 1956; Pipa and Cook, 1958). Later studies with the electron microscope have
confirmed the collagenous nature of the tissues in the various orders (Ashhurst and Chapman, 1961; Baccetti, 1961a, b; Hess, 1958; Smith and Wigglesworth, 1959). The collagen fibrils from most of the orders display banding with axial periodicities within the range of the periodicities found in mature vertebrate collagen fibrils, but, in the Coleoptera and Lepidoptera, the banding may have a periodicity between 15 and 20 m and be rather obscure (Baccetti, 1961a, b).

The central nervous system of insects is surrounded by a sheath composed of a homogeneous layer of collagenous connective tissue and an underlying layer of sheath cells. During metamorphosis in the Lepidoptera, the larval neural lamella is completely broken down, and after the reorganization of the nervous system to its adult form, the new adult neural lamella is formed. In addition, the Lepidoptera are peculiar, since an extra mass of fibrous connective tissue is formed on the dorsal side of the abdominal region of the central nervous system at this time (Ashhurst and Richards, 1964a). This is a large area of connective tissue, compared with the thin layers usually found in insects, and hence it was used for cytological and histochemical studies of developing insect connective tissue (Ashhurst and Richards, 1964a, b). It was found that the sheath cells are responsible for the production of the collagenous connective tissues of the adult neural lamella and of the dorsal mass. Acid mucopolysaccharides are associated with this developing tissue. While these investigations suggested that there are some similarities between connective tissue formation in insects and vertebrates, the real problems of fibrillogenesis, such as the site of production of the collagen molecules and of the fibrils, can only be solved by the use of the electron microscope. This paper is an account of some observations with the electron microscope which were made during fibrillogenesis in the pupa of the wax-moth, Galleria mellonella.

Materials and Methods

The cultures of Galleria mellonella L. used in this study were maintained at a temperature of 35°C; at this temperature, the pupal stage lasts for 6 or 7 days. By frequent observations of last instar larvae in their cocoons, it was possible to determine the time of pupation of a number of larvae accurately to within 1 h. A series of pupae of the following ages was taken for study: 24 h, 48 h, 73 h, 84 h, 90 h, 96 h, 102 h, 108 h, 114 h, 120 h, 132 h, and 144 h. A number of nerve cords were dissected from adults less than 24 h old.

The tissues were fixed in 1% osmium tetroxide in veronal-acetate buffer, pH 7.3 to 7.4, with the addition of trace amounts of calcium and magnesium chlorides. Owing to the general state of the tissues within the pupa, it was very difficult to detect the abdominal part of the central nervous system in freshly dissected pupae. In order to avoid this difficulty, fixative was injected into the abdominal region of the pupae, which were then left for approximately 1 h at room temperature before dissection; the nervous system was then more easily seen. The abdominal portion of the nervous system was removed and
put into ice-cold fixative for 1½ to 2 h. The abdominal nerve cord of the adult was easily found, as it moves constantly because of the contractions of the ventral diaphragm, which, in the Lepidoptera, is attached to the abdominal part of the nerve cord. The abdominal region of the adult nervous system was removed and fixed in the ice-cold fixative for 1½ to 2 h. All the nerve cords were taken from the fixative into 70% ethanol, dehydrated to absolute ethanol, and then stained overnight with 1% phosphotungstic acid in absolute ethanol. The nerve cords were embedded in araldite.

Some adult nerve cords were treated with hyaluronidase to try to remove some of the mucopolysaccharide which adheres to the connective-tissue fibrils. The following schedules were tried:

1. Nerve cords were fixed in the osmium fixative for 1 h, then transferred, after washing, to hyaluronidase at 37° C for either 3½ or 12 h. The hyaluronidase used was rondase: for the shorter incubation period it was at a concentration of approximately 200 IU per ml of 0.9% sodium chloride, and for the longer period at 1500 IU per ml of saline.

2. One nerve cord was incubated in hyaluronidase (rondase, 200 IU per ml of saline) for 4½ h at 37° C. After washing, it was fixed in the osmium fixative for 1 h.

3. Nerve cords were fixed in formaldehyde-saline overnight. After washing, they were transferred to hyaluronidase for 4½ h (rondase, approximately 400 IU per ml of saline) or for 18 h (1500 IU per ml of saline) at 37° C. The nerve cords were again washed and put into the osmium fixative for 1 h. The nerve cords, after the above treatments, were dehydrated in graded ethanols, passed through propylene oxide, and embedded in araldite.

Thin sections were cut on a Huxley ultra-microtome and were examined with a Siemens Elmiskop I electron microscope. Although the tissues had been stained in bulk in phosphotungstic acid, some of the thin sections were further stained in either lead citrate (Reynolds, 1963), 2% uranyl acetate in methanol, vanadium sulphate, or vanadatomolybdate (Callahan and Horner, 1964).

Some 2 μ sections of certain stages were cut and tested for the presence of acid and neutral mucopolysaccharides with toluidine blue and the periodic acid/Schiff test.

**Results**

It is known from a previous study of *Galleria mellonella* (Ashhurst and Richards, 1964a) that after the larval neural lamella has been broken down in the first 24 h after pupation, the sheath cells form a layer around the whole central nervous system and subsequently, in the abdominal region, they divide to form an extra pile of cells on the dorsal side of the nerve cord. A new neural lamella is visible with the light microscope around the whole nerve cord and the dorsal mass of cells by 72 h after pupation, and soon afterwards areas of fibrous connective tissue appear between the cells of the dorsal mass.
In electron micrographs, however, it was found that fibrillogenesis starts earlier than was at first thought: areas of fibrous tissue are visible between the cells in a 73 h pupa (fig. 1), but, while the neural lamella is clearly seen in a Masson preparation of this stage, it is not clearly differentiated in electron micrographs (fig. 6, A). In addition, it was thought from the earlier study that connective tissue formation was completed by 120 h after pupation, but the electron microscope evidence suggests that it proceeds until the emergence of the adult. Most of the nerve cords prepared for this study were from pupae ranging from 73 to 144 h old, since it had been deduced from the light microscope study that connective tissue formation is at its maximum during this time. A few nerve cords were obtained from pupae less than 72 h old. The observations were mostly made on the older specimens.

In the pupa, the main role of the sheath cells is that of connective tissue production and hence, at this time, these cells are fibroblasts. In the earlier study it was seen that at the beginning of the pupal stage, the sheath cells become vacuolated and enlarged. These vacuoles could not be characterized histochemically (Ashhurst and Richards, 1964a, b).

While the sheath cells are fulfilling their role as fibroblasts, there is little change in the cytoplasmic inclusions apart from variations in the number of certain vesicles, which increase to a maximum in the 108 h pupa, and then decline to very few in the adult. The total volume of the cytoplasm is being reduced steadily by the increasing fibrous areas. In the early stages before fibrillogenesis has advanced, the dorsal mass on the nerve cord is composed entirely of cells with no intercellular spaces, other than a narrow gap between the adjacent plasma membranes. The production of the fibrous areas, which in the later stages are quite extensive, is at the expense of the cytoplasm of the sheath cells.

The most characteristic inclusions of the moth fibroblasts are large round vesicles bounded by a single membrane, which encloses an amorphous material, more electron-dense than the surrounding cytoplasm. In the 73 h pupa these vesicles are not clearly in association with any other membranous components of the cytoplasm, but the nature of the vesicles becomes clear when micrographs of the later stages are examined. Fig. 2, A shows a typical aggregation of cisternae and vesicles found in the cells from a pupa 90 h old. The majority of the cisternae are roughly parallel to each other, though the arrangement is here not so regular as that seen in many other cells. Their outer membrane is covered by granules, which are within the size-range of ribosomes. These cisternae, therefore, form part of the granular endoplasmic reticulum of the cell. In fig. 2, A, many of the vesicles previously described

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**Fig. 1 (plate).** A low-magnification electron micrograph showing an area of the cells in the dorsal mass of the abdominal region of the central nervous system from a pupa 73 h old. A few vesicles (v) of the endoplasmic reticulum, mitochondria (m), and a few small intracellular fibrils (f) can be seen in the cytoplasm. Small areas of fibrous connective tissue (ct) are present. The plasma membranes (pm) divide the cells, but the membrane is not so clear near the fibrous connective tissue (arrows).
are present around the periphery of the cisternae, and in places their limiting membranes are seen to be continuous with the cisternal membranes. In addition, some of the cisternae appear to enclose a material of similar electron density to that in the vesicles, and in some areas, the cisternae appear to be enlarging. The evidence thus suggests that the vesicles are dilatations of the granular endoplasmic reticulum, which have lost most of their ribosomes. The material within the vesicles and cisternae is presumably a product of the synthetic activity of the endoplasmic reticulum. In general it has an amorphous appearance, but occasionally it appears to be slightly fibrous.

The formation of the vesicles continues and in fig. 2, B a rather extreme example is shown. This is a cell from a 108 h pupa in which the cytoplasm is completely filled by intercommunicating vesicles. No cisternae can be seen. The double nuclear membrane is very clear in this micrograph and in places the outer of the two membranes is continuous with the membranes of the vesicles. The outer nuclear membrane is usually considered to be a part of the endoplasmic reticulum and so the continuity between the outer nuclear membrane and the membrane of the vesicles may be taken as further evidence that the vesicles are part of the endoplasmic reticulum.

Throughout the rest of the pupal stage, the number of vesicles gradually decreases. Some typical cells from a pupa 144 h old are seen in fig. 3. The cytoplasm contains a few vesicles, but no other endoplasmic reticulum. The membrane around the vesicles may be disrupted in places. In the cells from an adult nerve cord, vesicles are rarely seen; the few which are present have a poorly defined membrane (fig. 5).

The increase in the numbers of vesicles within the cells appears to correspond with the times when the fibrous connective tissue is being laid down. Fibre production is probably at a maximum in the 108 h pupa, when the cells are packed with vesicles, and there is a subsequent decline towards the end of the pupal stage. It appears very probable that the vesicles and their contents are intimately concerned with the production of the fibrous connective tissues.

While there have been considerable differences of opinion about the intracellular or extra-cellular nature of aggregations of filaments near the plasma membrane of mammalian fibroblasts, there is no doubt about the intracellular location of fibrils in the fibroblasts of *Galleria*. Fibrils are seen rather sporadically in the cells during the early stages of fibre production, but in cells from a 144 h pupa and in adult cells they are seen very frequently and sometimes in large numbers. Their intracellular nature in micrographs such as fig. 4, A cannot be disputed, since they are clearly seen to be situated between the plasma membrane of the cell and the nucleus. In fig. 4, B a group of fibrils is seen in

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**Fig. 2 (plate).** A, the micrograph shows part of a fibroblast from a 90 h pupa, with a typical aggregation of cisternae (c) with attached ribosomes, and vesicles (v) of the endoplasmic reticulum. The membranes of the cisternae and vesicles are continuous (arrows).

B, an electron micrograph of a fibroblast from a pupa 108 h old, showing the great development of the vesicles (v) of the endoplasmic reticulum. The continuity between the membranes of the vesicles and the outer nuclear membrane can be seen (arrows).
transverse section with several mitochondria among them. In such sections the fibrils appear to have a dense cortex and much less dense core; their diameter is between 12 and 20 µm. Longitudinal sections of fibrils also suggest that the cortex is more electron-dense than the interior of the fibril. Very occasionally, indications of banding can be seen in the fibrils (arrows, fig. 4, A); the periodicity is very variable, being between 12 and 20 µm. In favourable sections, fibrils up to 1 µm in length may be seen.

It seems probable that the expulsion of these fibrils from the cytoplasm to the areas of fibrous connective tissue involves the breakdown of the plasma membrane. In the early stages of fibre production (e.g. fig. 1), the plasma membranes of the cells are clearly seen. The plasma membrane in the later stages (fig. 5) is conspicuous where the cytoplasm of two cells is adjacent, but where the cytoplasm of a cell borders on an area of fibrous tissue, its plasma membrane is no longer present as an intact membrane. It is thus suggested that after their formation, the fibrils move towards the periphery of the cell and both the fibrils and their surrounding cytoplasm become incorporated into the fibrous tissue. It was mentioned earlier that the area of fibrous tissue is increased at the expense of the cytoplasm of the cells.

The fibrous areas do not have the appearance of typical collagenous connective tissue. Although they are obviously fibrous, the individual fibrils are obscured by an amorphous material which surrounds them. They are covered with this material to such an extent that it would seem at first sight that they bear little relation to the intra-cytoplasmic fibrils. However, very occasionally an imperfectly surrounded fibril, such as that in fig. 4, C (arrow) is observed. The ground substance of the fibrous areas contains acid and neutral mucopolysaccharides (Ashhurst and Richards, 1964b) and these substances can be detected in the material prepared for electron microscopy. It is thought that the amorphous material surrounding the fibrils in *Galleria* is composed of the acid and neutral mucopolysaccharides of the ground substance. The close adherence of this material to the individual fibrils may perhaps be a result of the fixation and embedding procedures. Treatment with hyaluronidase during the preparation of the material had some effect on the amorphous material, but failed to remove it from the fibrils. In the later pupal stages and in the adult, bundles of the intracellular fibrils are frequently seen. Sometimes such a bundle appears to have lost its plasma membrane and to be in the process of acquiring the amorphous ground substance (fig. 4, D). The presence of large numbers of intracellular fibrils in adult cells (fig. 5) is difficult to explain. Throughout fibrillogenesis, both the protein of the fibrils and the mucopolysaccharides of the ground substance are being produced. The presence of intracellular fibrils in the adult may indicate that the production of the

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Fig. 1 (plate). A low-magnification micrograph showing an area of fibroblasts and fibrous connective tissue (ct) in a pupa 144 h old. The number of vesicles (v) is declining and their membranes are disrupted. Intracellular fibrils (f) can be seen among the mitochondria (m). The plasma membrane is not visible where the cytoplasm is adjacent to the fibrous connective tissue (arrows). The section is stained with vanadatomolybdate.
FIG. 3
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mucopolysaccharides ceases while collagen production is continuing until a later stage.

The lateral and ventral regions of the abdominal nervous system are surrounded by the neural lamella and a thin layer of sheath cells (fig. 6, B). The cells are very similar to those in the dorsal mass, but the fibrils and their surrounding matrix are much more densely packed.

In the histochemical study of the fibroblasts (Ashhurst and Richards, 1964b), glycogen was found in the cytoplasm. It has not been possible to identify deposits of glycogen in the micrographs. No special techniques for glycogen have been employed.

Discussion

The results described in this paper suggest the way in which fibrous connective tissue is laid down in a moth. While it is possible that the mechanism is different even in other orders of insects, it is interesting to compare some of the features of the moth fibroblasts and connective tissue with those of the vertebrate connective tissues that have been studied.

The most characteristic feature of moth fibroblasts is the abundance of large vesicles filled with a somewhat electron-dense material. Evidence has been given which suggests that these vesicles are in fact dilatations of the granular endoplasmic reticulum and that the amorphous material within them is a product of the endoplasmic reticulum. The similarity between the endoplasmic reticulum and its associated vesicles in the moth, and the endoplasmic reticulum in many vertebrate fibroblasts is striking. Fibroblasts with dilated endoplasmic reticulum have been described in amphibian skin (Weiss and Ferris, 1956), carrageenin granuloma in the guinea-pig (Chapman, 1961), skin of chick embryos (Gieseking, 1960), aorta of chick embryos (Karrer, 1960), skin of embryo mice (Kajikawa, 1961), regenerating tendon of the guinea-pig (Peach and others, 1961), and in skin wounds of the guinea-pig (Ross and Benditt, 1961). The contents of the swellings of the endoplasmic reticulum vary in the tissues mentioned above, but substances more electron-dense than the surrounding cytoplasm were described in the vesicles by Chapman (1961), Gieseking (1960), Kajikawa (1961), Karrer (1960), Peach and others.
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(1961), and Ross and Benditt (1961). All these authors found evidence to suggest that the intra-cisternal or vesicular material might be fibrous; Chapman and Kajikawa describe filaments 5 mμ in diameter. In Galleria the contents of the vesicles sometimes appear to be rather fibrous, but distinct filaments were not observed.

No Golgi membranes or vesicles could be identified in the micrographs of moth fibroblasts. In vertebrate fibroblasts they seldom achieve any prominence, but in the chondrocytes of the ear cartilage of the rabbit and the hind limb rudiments of embryo and new-born rats, the Golgi vesicles are well developed (Godman and Porter, 1960; Sheldon and Kimball, 1962). Fibrils with an axial periodicity of about 200 mμ were present in some Golgi vacuoles of the rabbit ear cartilage; Sheldon and Kimball suggest that this corresponds to the periodicity of fibrous long-spacing collagen.

The intracellular situation of some of the fibrils in moth connective tissue is indisputable, but in vertebrate fibroblasts the situation is not so clearly defined. Intracellular fibrils were not described in fibroblasts from aorta of chick embryos (Karrer, 1960), dermis of chick embryos (Porter and Pappas, 1959), and regenerating tendon of the guinea-pig (Peach and others, 1961), but they have been observed in many other fibroblasts, for example, in carrageenin granuloma of the guinea-pig (Chapman, 1961), the skin of chick and mouse embryos (Gieseking, 1960; Kajikawa, 1961), heart of chick embryos in tissue culture (Yardley and others, 1960), hind limbs of rat embryos (Godman and Porter, 1960), and in skin wounds of the guinea-pig (Ross and Benditt, 1961). These fibrils are usually unbanded and their diameters range between 2 and 10 mμ. The intracellular fibrils seen in Galleria fibroblasts are rather larger than the fibrils seen in the vertebrate cells, since they are between 12 and 20 mμ in diameter; in addition, they occasionally show indications of an axial periodicity between 12 and 20 mμ. While the intracellular fibrils of vertebrates are considered to represent a stage in the polymerization of the tropocollagen molecules, by which process the mature collagen fibrils are formed, it appears that in Galleria these fibrils represent the final stage in the polymerization of the collagen, since it seems that fibrils identical to these are present in the fibrous connective tissue.

The discontinuities in the plasma membrane of these moth fibroblasts in areas where the cytoplasm and fibrous tissue are adjacent have been described earlier. It is suggested that the fibrils formed within the cytoplasm of the cells migrate towards the periphery of the cytoplasm, where they become associated with the substances of the matrix, such as acid mucopolysaccharide and lipids.

Fig. 5 (plate). A low-magnification electron micrograph of part of the dorsal mass of the abdominal regional region of the nervous system of an adult moth. The cells are much smaller. Many mitochondria (m) are present in the cytoplasm, but only remnants of the vesicles (v) can be seen. Many transverse sections of the intracellular fibrils (f) occur in the cytoplasm. The plasma membranes (pm) are well defined between the cells, but are discontinuous next to the fibrous connective tissue (ct) which is now extensive (arrows). Part of one of the connectives of the nerve cord (nc) is present.
FIG. 6  
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(Ashhurst and Richards, 1964b). There appears to be no distinct time or method of extrusion from the cell. This is probably a result of the closely packed arrangement of the cells. In most vertebrate connective tissues the fibroblasts separate, leaving an intercellular matrix, before fibril formation begins (Chapman, 1961; Godman and Porter, 1960). Several studies have suggested that the plasma membrane may not always be intact in vertebrate fibroblasts (Chapman, 1961; Peach and others, 1961; Yardley and others, 1960). These regions of discontinuity of the plasma membrane are often associated with areas of intracellular filaments just below the plasma membrane. An apocrine form of secretion has been suggested by Chapman (1961), Gieseking (1960), and Yardley and others (1960). It is thought that an area of the cytoplasm containing filaments and the substances of the matrix is ‘pinched off’ from the cytoplasm. Alternatively, Karrer (1960) presents evidence that the vesicles of the endoplasmic reticulum may come to the plasma membrane and be extruded whole, or that the membrane of the endoplasmic reticulum and the plasma membrane fuse, expelling the contents of the endoplasmic reticulum into the intercellular spaces. There is no evidence for any of these mechanisms in Galleria; all require the presence of intercellular spaces, and none is present in the moth tissue.

The close packing of the cells and fibrous areas, together with the discontinuity of the plasma membrane, suggests that a form of true syncytium may occur in Galleria. It then becomes extremely difficult to determine with any certainty the intra- or extra-cellular status of the fibrous connective tissue.

The fibrous tissue in Galleria is unlike that found in any vertebrate tissues, but it is similar to that in the neural lamella of the coleopteran, Blaps gibba (Baccetti, 1961b). The mature fibrils are obscured, except in rare instances, by irregular masses of the ground substances. The immature fibrils are seen in the fibroblasts, and occasionally traces of banding with periodicity between 12 and 20 μm may be observed. Banding with a periodicity between 15 and 20 μm was seen in the collagen fibrils of the neural lamellae of two other Lepidoptera and a coleopteran by Baccetti (1961a). However, collagen fibrils with banding of periodicities within the range recorded for mature vertebrate collagen fibrils are known to be present in many other insects, such as cockroaches, locusts, and Rhodnius (Ashhurst and Chapman, 1961; Baccetti, 1961a; Hess, 1958; Smith and Wigglesworth, 1959). The size of the fibrils and the periodicity of their banding in Galleria are more comparable to those of immature vertebrate fibrils, which are of small diameter and have a banding periodicity between 20 and 30 μm (Gieseking, 1960; Jackson and Smith, 1957). The collagen fibrils from the byssus threads of molluscs and the cuticles of Ascaris and earthworms are all unbanded, though there is abundant chemical
evidence to support their collagenous nature (Bird, 1957; Bird and Deutsch, 1957; Gross and Piez, 1960; Jackson and others, 1953; Watson, 1958; Watson and Silvester, 1959). The collagenous nature of the fibrils in Galleria was determined earlier on the histochemical reactions of the tissue and the presence of hydroxyproline in hydrolysates of nerve cords (Ashhurst and Richards, 1964). The apparent lack of banding in most collagen fibrils of the moth is probably due to the arrangement of the tropocollagen molecules. These molecules are approximately 280 mµ long and 1.4 mµ wide, and their regularly staggered arrangement with respect to adjacent molecules along the collagen fibril is responsible for the regular 64 mµ banding of the typical vertebrate fibril (Schmitt, 1959). Maser and Rice (1963) have suggested that collagen molecules of earthworm cuticle might be dimers of tropocollagen-like particles and that this might have an effect on the banding. The similarity in the banding of collagen fibrils in the moth and the immature vertebrate fibrils, and the fact that no further aggregation of molecules occurs within the fibrous tissue, as it does in the extracellular matrix of vertebrates, may perhaps indicate that mature moth fibrils are in an immature state compared to the fibrils exhibiting typical 64 mµ banding. Nothing is yet known of the chemical and physical properties of moth collagen; studies of this nature on insect collagens are severely hampered by the very small amounts of tissue available.

The fibrils in Galleria are also unusual in that they appear to be hollow. Curran and Clark (1963), however, suggest that in granuloma tissue, the collagen fibrils are produced by thin filaments adhering to electron-translucent rods. The mature fibrils retain their translucent core, 30 to 40 mµ in diameter, which is thought to be filled with mucopolysaccharide. At present there is no evidence to suggest what might be present in the moth fibrils, but mucopolysaccharides would appear to be the most probable substances.

Mucopolysaccharides, particularly the sulphated mucopolysaccharides, are closely associated with collagenous connective tissues. They are thought to be important in the stabilization of collagen molecules (Jackson, 1954), but their presence is probably not essential for collagen formation, since the concentration of chondroitin sulphate in wound tissue is less than that in the surrounding skin (Jackson and others, 1960). In some developing vertebrate connective tissues, glucosamine can be detected early in development, but galactosamine is not present until after the collagen fibrils have been formed (Bouek and others, 1959). These amino-sugars are products of the hydrolysis of hyaluronic acid and of chondroitin sulphate, respectively. Later, as the connective tissue matures, the amount of acid mucopolysaccharide present usually decreases (Williams, 1957). In some tissues, for example cornea, a high concentration of acid mucopolysaccharide persists and this has been correlated with the incidence of fibrils with small diameters, i.e. less than 30 mµ (van den Hooff, 1957). Further evidence for the correlation between a high content of acid mucopolysaccharide and the presence of fibrils of small diameter was found by Wood (1960). A mechanism of fibril formation in two stages was described (Wood, 1960a): in the first stage, the collagen molecules form aggregates,
which in the second stage grow by the accretion of soluble collagen molecules from the surrounding medium. The introduction of chondroitin sulphate A into the system accelerated the first stage, resulting in the production of fibrils of small diameter (Wood, 1960). Invertebrate collagens typically contain a greater amount of mucopolysaccharide than vertebrate collagens (Gross and Piez, 1960), and in Galleria, no decrease in the acid mucopolysaccharide of the fibrous tissue could be detected as the tissues aged (Ashhurst and Richards, 1964). It is thought that the acid mucopolysaccharide is most probably a form of chondroitin sulphate. It is tempting to correlate the occurrence of fibrils of small diameter in Galleria with the presence of this acid mucopolysaccharide, especially as in insects, such as locusts and cockroaches, which have typical collagen fibrils in the neural lamella, acid mucopolysaccharides are not present in detectable amounts in the adult tissues (Ashhurst, 1959, 1961).

There is good evidence that the sulphated mucopolysaccharide, chondroitin sulphate, of vertebrate connective tissues is produced by the fibroblasts and chondrocytes (Dziewiatkowski, 1962; Mancini and others, 1961; Thorp and Dorfman, 1963). It is assumed that the same is true in Galleria, but while it is probable that mucopolysaccharides are produced by the granular endoplasmic reticulum, there is, as yet, no direct evidence for this. On the other hand, it is known that the first-formed collagen, which is soluble in neutral salt solution (Jackson and Bentley, 1960), is found, after ultracentrifugation of carrageenin granuloma, in the microsome fraction; this result indicates that the microsomes are the site of the production of the collagen molecules (Lowther and others, 1961). The rather dense contents of the endoplasmic reticular vesicles in Galleria fibroblasts are thought to be either the precursors of, or the actual tropocollagen molecules produced by, the ribosomes on the endoplasmic reticulum. It would seem probable that if the cell also produces acid mucopolysaccharides, these too are present in the vesicles.

While the exact role of the sheath cells in the maintenance of the ionic environment inside the nervous system is not yet determined fully (Treherne, 1962), it might seem strange that cells which serve a function in relation to ionic balance in the larva and adult should become fibroblasts in the pupa. It appears, however, that in the vertebrates, collagen production is not necessarily confined to typical fibroblasts: Nathaniel and Pease (1963) reported that Schwann cells could produce collagen, and Pease and Molinari (1960) concluded that smooth muscle cells must produce the collagen found in certain areas of the pial vessels of the brain of cats and monkeys. Some fibroblasts of the tunica media of developing aorta lose their inclusions in the later stages of fibrillogenesis and appear to transform into smooth muscle cells (Karrer, 1960). Thus it seems possible that the sheath cells play a dual role.

In the foregoing discussion, the main theme has been the comparison of insect and vertebrate fibrillogenesis. The similarities between the inclusions of the fibroblasts of the two phyla are considerable, and it is very probable that the site of the production of the collagen molecules is the same. The main differences occur in the later elaboration of the collagen molecules into the
fibrils and in the organization of the fibrous tissues. It is interesting that in the leech, where the collagen fibrils are ‘shredded off’ from the plasma membrane of the fibroblast (Bradbury and Meek, 1958), fibrillogenesis appears to be markedly different from that of the moth and vertebrates.

This research was started during the tenure of a N.A.T.O. Fellowship. I am grateful to the Agricultural Research Council for providing financial support for its completion. I should like to thank Dr. D. Lacy and Professor J. W. S. Pringle, F.R.S., for providing accommodation and facilities for me in their respective Departments in London and Oxford. My thanks are also due to Dr. A. G. M. Weddell for allowing me to use the Siemens electron microscope, and to Miss E. G. M. Collins for making the prints for publication. Finally, I should like to express my gratitude to Dr. J. R. Baker, F.R.S., for his unfailing help, interest, and encouragement.

References

— 1961. Ibid., 102, 455.
— 1964b. Ibid., 114, 237.
— 1956, Ibid., 41, 259.
— 1961a. Ibid., 46, 1.
Postscript. Microtubules, with dimensions similar to those of the intracellular fibrils in *Galleria*, have recently been described in a number of protozoan, invertebrate, vertebrate, and plant cells. These are thought to be part of the mitotic spindle in *Pelomyxa* and *Hydra* (Roth and Daniels, 1962; Slautterback, 1963); they are also present around the nematocyst in *Hydra*. In plant cells they are associated with developing cell walls (Hepler and Newcomb, 1964; Ledbetter and Porter, 1963). They are present in most vertebrate cells after fixation in glutaraldehyde (Behnke, 1964; Sandborn and others, 1964). None of these microtubules is banded in such a way as to resemble the intracellular fibrils in *Galleria*. The microtubules in *Pelomyxa* show indications of banding with a periodicity of 6 mμ; the others are not banded. The fibroblasts in *Galleria* are not dividing. The increase in the number of intracellular fibrils in *Galleria* during the production of the fibrous tissue suggests that they are intimately concerned in fibrillogenesis.