THE FLIGHT MUSCLES OF INSECTS—THEIR ANATOMY AND HISTOLOGY; WITH SOME OBSERVATIONS ON THE STRUCTURE OF STRIATED MUSCLE IN GENERAL

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[Plates 17 to 32]

CONTENTS

The primitive insect, in acquiring the power of flight, must initially have used the existing thoracic musculature to produce the deformation of the thoracic wall required to move the wings: the dorso-ventral muscles inserted on the leg base were, even without change of attachments, used in the capacity of indirect wing levators, for by contracting in mass they depressed the tergal wall and so raised the wing; the pleural (epipleural) muscles, by pulling on the wing, became 'direct' wing depressors; subsequently the development of phragmas enabled the longitudinal tergal muscles to function as weak indirect wing depressors, and to become, eventually, even the principal wing depressors. Such, at least, is the evidence derived from Orthoptera.

In higher insects (but not Lepidoptera) evolution of the flying mechanism entailed the development of an increasingly rapid wing beat, leading in Hymenoptera and Diptera to very high-frequency wing vibration with a minimum of thoracic deformation; in the wing musculature it led to the evolution of a completely novel type of muscle in which high-frequency unfused con-

traction, under almost isometric conditions, replaced low-frequency tetanic shortening. A thoracic muscle, functioning in its original capacity as leg muscle, can only to a restricted degree accept the additional role of wing vibrator, and must have become increasingly useless in its initial role as it developed its new function. In Homoptera, which have been examined in some detail, such a crisis seems to have occurred: the cicadas and various fulgorids have to a surprising degree diverted muscles to the exclusive role of wing vibrators; others, like jassids, have distributed the two functions more evenly in different muscles, and there are signs (in Cercopidae) that even new muscles are generated from existing muscles to act as wing vibrators. A surprising feature in some Homoptera is the co-opting of certain abdominal muscles into the flying musculature, to meet the deficiency of metathoracic muscles. In the Diptera, the only other order examined, the wing vibrators are reduced to a small number; most of the dorso-ventral muscles associated with the leg base disappear, and the tergal wall of the mesothorax is an almost exclusive, in some forms even exclusive, alinotum. Here departure from the primitive orthopteran condition is at its greatest. There is, on the other hand, a strong development of new steering muscles in Diptera.

Change in physiological property of the muscle tissue is attended by changes in the histology of its fibres. The orthodox view that the wing musculature of higher insects is composed of fibres strictly comparable with those of other muscle is upheld, but only after a re-assessment of the latter. It appears that in normal arthropod fibre the fibrils are actually composed of a small number of contractile myofibrils bound into a unit by 'ground substance' in which they are embedded; for this compound fibril the term 'sarcostyle' is adopted in this new sense. In the higher, and even intermediate groups, in which the tendency is toward high-frequency isometric contraction, the sarcostyles undergo remarkable thickening; these are the coarse fibrils first described by von Siebold (1848) and now well known for all the higher orders where the wing beat is rapid. In the sarcoplasm the most noteworthy change is the disappearance of the Cohnheim reticulum even within Orthoptera, and in the higher groups a liquifying of the sarcoplasm, presumably to promote mobility of the tissue; it is to the liquid sarcoplasm that the tissue owes its unique tendency to dissociate into fibrils. With these changes is associated an increased development of sarcosomes, which enlarge and adopt a direct relation to the cross-striation. Of the cross-membranes a *Z*-membrane completely transecting the fibre is an integral component of normal striated muscle fibre; a similar *M*-membrane is certainly present in vertebrate fibres, but (through lack of specific technique?) can only in exceptional cases be seen in arthropod muscle. Both membranes have appeared in the wing-muscle fibres, even in coarsely fibrillated (Siebold) muscle. It seems to be the cross-membranes, usually helicoidally disposed, that impose on the fibril bundle its pattern of cross-striation. The last criterion for assessing the presence of a muscle fibre is a sarcolemma; in all cases examined such a membrane has appeared, even in species in which its presence is currently denied.

In most blattids the wing-muscle fibres do not show any recognizable adaptation to flight; in other primitive Orthoptera the Cohnheim reticulum is retained, and the only perceptible adaptation to flight is an inconstant increase of sarcosome content, and the penetration of a few blindly ending tracheae into the fibres. Yet in Acridiidae a rich sarcosome content has developed, and there is an abundance of intracellular tracheae leading even to closed net formation. In Homoptera, where flight is more highly developed, the musculature seems to be transitional to that of higher insects; the sarcostyles commonly confer a special pattern on the cross-cut fibre, and may be thin or of the thick (Siebold) type, but always respond well to electric stimulation; sarcosomes are abundant and the tracheae form closed nets to a degree far beyond those found in Orthoptera. In the Diptera, the only higher order examined, shortening of sarcostyles in response to electric stimulation is restricted; sarcosomes are always abundant, and the tracheae always form closed nets within the fibre. Different families are distinguished by the fibre pattern of their wing muscles. In some the fibres are numerous; in most they are few and generally constant in number, and wherever the insect is large this leads to giant fibre formation. Development of giant fibres is attended in Diptera by elaboration of their internal tracheae beyond anything found in other insects; usually there is a development of a highly elaborated pattern sometimes even having a relation to the cross-striation.

In Homoptera, as in Orthoptera, the motor-nerve fibres end by Doyère end-organs just under the sarcolemma; in various Diptera the innervation has been found to be by numerous nerve twigs all along the fibre, ending, not just under the sarcolemma, but deeply within the interior of the fibre.

Throughout the Orthoptera enlargement of the flight muscles is attended by fibre proliferation; specific flying muscles develop by repeated division of rudimentary muscle fibres; muscles that function in double capacity enlarge with growth of the nymph, the most intense fibre cleavage being apparently without effect on the functioning of the tissue. Only in the last instar do the fibres acquire the special histological characters that adapt them to flight. In Homoptera, also, there is evidence of fibre cleavage. In cicadas the entire wing musculature is generated by the repeated division of a few rudimentary fibres. In various other Homoptera, where the wing musculature is composed of many fibres, there is cleavage either of functional nymphal fibres (cercopids) or of rudimentary fibres arising from myoblasts in the minute nymph; only in jassids with small fibre number is cleavage suppressed. But in all these forms (except cicadas) a new histogenetic process soon supervenes, formerly free myoblasts becoming incorporated in a remarkable manner into the young fibres; the myoblasts prolong, either singly or in chains, alongside the enlarging fibres, successively adding to the latter a new fibril with investing sarcoplasm, and with attendant nuclei, and these new fibrils may or may not be subject to fibril cleavage. Among the Homoptera the most specialized process is that found in small jassids, where each myoblast contributes to the fibre a single fibril. In Homoptera the young fibres are usually, from the beginning, faintly cross-striated, and to the existing pattern the newly added fibrils accommodate themselves. In Diptera the myogenesis is concentrated in the pupal period, in contrast to that of Homoptera, where it occupies most of the nymphal period. It is a process in which multitudes of myoblasts co-operate, each apparently supplying a single fibril, as in jassids; but cross-striation is here deferred till the full fibril number has appeared. The meaning of this type of myogenesis is unknown; but it emphasizes the fact that the formation of a striated muscle fibre is a more plastic process than we have hitherto suspected.

Preface

The work to be described in this memoir has had its origin in some observations that I made, over thirty years ago, while examining the processes undergone by the tissues of an insect during metamorphosis. They relate to the histogenesis of the wing muscles, and disclosed a form of myogenesis, still unknown to histologists, but so novel in character as to merit more detailed examination.

I had originally intended that the present work should be a brief study to amplify the earlier results; but it soon became apparent that the subject had greater scope than was at first suspected. The work has accordingly grown into a more ambitious attempt to understand the structural evolution of the wing musculature, as the flying mechanism became perfected.

In the ancient winged insect it was the old thoracic musculature that was first pressed into the service of the new function of crude flight. As the higher orders developed, the flying mechanism became perfected by the evolution of an entirely novel and indeed almost unique mechanism, that of high-frequency wing vibration, which reaches the summit of its achievement in the higher Diptera and Hymenoptera. It is the purpose of the present work to examine the changes in the musculature that have made this possible, and particularly the structural adjustments by which the striated muscle fibre has accommodated itself to these specialized and exacting demands.

Introduction

More than a hundred years ago von Siebold (1848) first drew attention to some unusual features in the texture of the great flying muscles of insects; for he had discovered the remarkable coarse fibrils of which they are composed, and into which they dissociate when the tissue is disrupted. The gross anatomy and mechanism of the wing musculature

were already well known, having been described by Chabrier, Jurine and, in minute detail, by Straus-Durckheim in his celebrated work on *Melolontha*; the principal flying muscles, except in Odonata, had no direct connexion with the wing bases, but were attached to the walls of the thorax alone, by the deformation of which they exerted their effects 'indirectly' on the wings. But to von Siebold was reserved the discovery of their peculiar histology—their construction out of coarse, easily dissociated fibrils—and this has earned for them the name 'fibrilläre Muskeln' or 'Siebolds Muskeln' (Kölliker 1888), for which the English term 'fibrillary' or 'fibrous' muscle is sometimes used.

The widespread prevalence, among insects, of this type of flying muscle is now well known. But already in 1853 Aubert had shown that it was not of general occurrence, for it was not present in Orthoptera, nor in Odonata, the latter having a peculiar form of muscle in which the fibrils had the form of strap-like bands ('lamellar' muscle of later writers). To the histologists of that time the distinction between the two types of muscle lay in the supposed absence of true muscle fibres in the Siebold type of wing muscle, the fibres having escaped detection because of their tendency to disrupt into their component coarse fibrils. That these fibrils were often bound together by tracheae into bundles was recognized; but in the descriptions of such writers as Aubert (1853), Merkel (1872), Sachs (1872), Wagener (1873), Biedermann (1876), Limbeck (1885), and Ciaccio (1887) there is always a distinction, either implicit or explicit, between muscles that are composed of fibres, and those that are composed of fibrils; to quote Limbeck: 'the thorax muscles do not consist of muscle fibres, comparable with those of vertebrates, but are constructed of fibrils, of which a large number are always held together by a system of tracheal nets, and so appear as an analogue of a muscle fibre.' On the other hand, Kölliker (1888), Cajal (1890) and Schäfer (1891) speak explicitly of 'muscle fibres'. But as late as 1903 Münch was impelled to write that the still prevalent view as to the absence of muscle fibres in the thorax muscles was erroneous, there being 'no ground whatever for regarding these muscles as fundamentally different from others'.

Yet even a cursory inspection of the two types of muscle reveals pronounced differences between them. On general grounds it would seem that the coarse fibrillar (Siebold's) muscle is merely a highly specialized form of trunk muscle, and that in some way its very exceptional structure is bound up with its very exceptional physiological properties. In conformity with this the comparative anatomical studies of Voss (1905), Snodgrass (1935), Weber (1933), Maki (1938) and others have shown that the wing musculature of higher insects has indeed arisen by enlargement and specialization of the existing trunk musculature, and that it is not a musculature *sui generis*. Yet in regard to the histology of the muscle, real gradations between the two have not been recognized, and a strict comparison between them still presents difficulty. Long ago Rollet (1885) wrote: 'the flying muscles of insects are so essentially different in their structure from other cross-striated muscles of these animals, that observations on the one permit of no conclusions in regard to the other'; yet Schäfer (1891) proclaimed that 'the essential correspondence in structure between the two kinds of fibres cannot be overlooked when they are rigidly compared with one another'. The controversy is long over, and the essential similarity of the two kinds of muscle is now accepted with little dissent. But the difficulties remain; indeed, some of them have not hitherto been known.

In a re-examination of this question we face the truly surprising fact that the histology even of ordinary striated muscle is still a matter of contention, and not only with respect to minute detail, but even as regards some of its basic features. It has proved indeed a most intractable tissue. Over a hundred years ago William Bowman (1840) wrote that its 'form and composition have been objects of continual dispute.... The improvements which have taken place in the construction of microscopes appear indeed to have only afforded grounds for new difference of opinion'; and a succession of observers, to whom we owe much of our knowledge regarding its structure—Dobie, Engelmann, Rollet, Schäfer, Merkel, Hürthle, Heidenhain—have in turn remarked on this. Figure 20, plate 17, shows the bewildering structure that the fresh tissue may, at times, present to the observer. How the optical effects change with depth of focus is familiar to all histologists; and the contractility and fibrous structure of the tissue render it unusually prone to distortion. Moreover, fixation reveals a wealth of structural detail, of which the living tissue may give little evidence. We underestimate the value of a fixed preparation if we dismiss it, without further inquiry, as mere artifact.

Earlier work on the structure of muscle was summarized by Heidenhain in 1898, and later in his critical survey in *Plasma and Zelle* (1911), and these remain the only adequate reviews of the extensive literature on the subject. They are, however, marred by undue speculation on points that have become significant for an understanding of the basic structure of muscle tissue, and which are therefore of importance in comparing insect wing muscle with the less specialized type of muscle fibre. In the following introductory paragraphs, therefore, in which such a comparison is drawn, I have found it necessary to present a critical appraisal of much of the histology even of 'normal' insect muscle fibre. From the comparison there will emerge the various questions, which it is the purpose of the present work to answer.

It is important, in assessing the histology of insect muscle, that we do not extend to the latter, without full inquiry, data obtained from an intensive study of vertebrate muscle; for if current views on the derivation of Arthropoda from segmented worms are well founded, then striated muscle within this group must have arisen independently of that of vertebrates. Yet the resemblances between the two are certainly very remarkable, and emphasize the need to determine the functional significance of all structural detail.

A. *Sarcolemma*

In leg or trunk muscle of insects, the presence of a sarcolemma is now generally accepted. In fixed preparations it is often hard to see, especially in the longitudinally cut fibre; but in sections cut from the fresh frozen tissue it is always visible as a sharply defined smooth investing membrane (figures 31, 36, 41, 44, 45, plates 18 and 19). In orthopteran wing muscle it has been described by Holmgren (1908), Kielich (1918) and Jordan (1919), and is present also, apparently, in that of Lepidoptera (van Gehuchten 1886; Kölliker 1888; Holmgren 1908). But in the coarsely fibrillated (Siebold) muscle, Kölliker (1888) failed to see a sarcolemma, the fibres being ensheathed only by tracheal tubes; but Cajal (1890), Schäfer (1891), Münch (1903), Holmgren (1908), Kielich (1918) and Ciaccio (1940) report a sarcolemma, though according to recent work it is absent in the bee (Kielich 1918; Snodgrass 1925; Morison 1928) and in *Drosophila* (Williams & Williams 1943). Whether the

sarcolemma, where seen, is identical with Kölliker's tracheal sheath is uncertain. Bütschli & Schewiakoff (1891), who find a sarcolemma in *Prionus*, do indeed describe its formation out of muscle protoplasm, but opposed to this is the statement of Holmgren (1908) that the sarcolemma is partly composed 'of the protoplasm of the tracheae, spread out as a web between the individual tracheal tubes', describing it in a later paper (1910) as 'a product of the tracheal system, and not a differentiation of the muscle fibre itself'. It will be shown below that in all species examined, including bee and *Drosophila*, a sarcolemma is present, and that, moreover, it arises as a plasma membrane from the muscle fibre itself, and not from the tracheal investment.

With the recognition of the sarcolemma we affirm the existence of true muscle fibres even in the wing musculature; for a bundle of fibrils thus delimited fulfils the one essential criterion of a muscle fibre. But in some insects, as will be described below, the fibres thus delimited are of giant dimensions, with a wonderfully organized system of internal tracheae, far surpassing in complexity of structure any other known type of muscle fibre. It seems to be these huge muscle fibres, not easily recognized as such, that have led some writers to refer to them as 'muscle masses'.

As long ago as 1859 Amici saw that the sarcolemma displayed a peculiar 'festooning' at its periodic attachments to the cross-membranes. A succession of observers (Krause 1869; Engelmann 1873; W. Rutherford 1897; Marceau 1903; Holmgren 1908; Heidenhain 1911; Prenant 1911; Baldwin 1913; Jordan 1917; Morison 1928; von Boga 1937; Ciaccio 1938) confirm this. It is one of the most readily observed features of the muscle fibre, especially in arthropod muscle, and it is therefore strange that Barer (1948), in a recent review, should doubt such a connexion. The festooning is recognizable in the fresh fibre (figure 62*a*, plate 20), while the connexion with the cross-membranes is visible in any good longitudinal section, provided the sarcolemma is clearly visible (figures 21, 23, 37, 38, plates 17 and 18), and it is especially conspicuous if the fibre is contracted. It will be shown below that such a connexion between sarcolemma and cross-membranes is present also in coarsely fibrillated wing muscle, where its presence has not hitherto been reported.

B. *The fibrillary structure of muscle*

That there is a close analogy between the thick fibrils of specialized wing muscle, and the fine fibrils of leg muscle, we infer from the fact that both are the ultimate units into which the respective fibres can be dissociated; and as long ago as 1850 Kölliker had adduced the existence of the coarse fibrils, easily seen in the fresh tissue, as evidence of the fibrillar structure of muscle in general, which, since its description by Schwann, had already become controversial. With the discovery of the 'muscle columns' (Säulchen) by Kölliker in 1866, this simple solution of the problem of the coarse fibrils seemed less certain; since then, also, there appears much confusion in the literature concerning the criteria for distinguishing true fibrils from muscle columns. A clarification of this point is of much importance for the present work.

(i) *Muscle columns and Cohnheim areas*

The discovery of the muscle columns grew out of the observation of Cohnheim (1865) that thin cross-sections of frozen, unfixed and apparently still living muscle fibres, when

examined in saline, display a mosaic of minute areas that seem to be held together by a network of 'interstitial substance'. It has become the custom, in histological treatises, to misrepresent these Cohnheim areas as cross-sections of large bundles of fibrils; but Cohnheim himself gives their dimensions as not more than 2 to 5μ in the frog, even less in the mammal, but larger in the crayfish. The peculiar pattern displayed by a cross-section of a fresh muscle fibre, prepared as directed by Cohnheim, is shown in figure 18, plate 17 (leg-muscle fibre of rat); reference to the attached scale of magnification, which is a Grayson ruling in which one division is about 1μ, will give the dimensions of the Cohnheim areas. That the areas cannot simply be cross-sections of single fibrils, or of small or large bundles of fibrils, is at once evident by comparison of the frozen section with a section of the fixed tissue, in which the fibrils are stained (figure 19, plate 17). In longitudinally cut fibres, as Kölliker (1866) saw, the network of the cross-section assumes the form of fine longitudinal streaks of dark interstitial substance, often containing visible granules, and it is these that demarcate the substance of the fibre into muscle columns. These streaks of interstitial substance are only dimly visible in the completely fresh muscle fibre, but become more distinct after immersion in physiological saline, giving to the fibre a markedly fibrillar appearance (figure 20, plate 17). They are indeed apt to be mistaken for fibrils, unless frozen sections and fixed preparations are compared (cf. figures 21, 22, plate 17). If the frozen section in figure 20 is carefully examined, the fibrils, indeed, cannot be seen; but there are visible transverse rows of minute dots between the Q-bands, and as Schäfer (1891) saw, these dots are specks of sarcoplasm between adjacent fibrils. It will at once be seen that the fibrils, whose presence they betray, outnumber the muscle columns delimited by the streaks of interstitial substance. It will further be seen, from figure 20, that the streaks of interstitial substance do not pass the whole length of the fibre, so that adjacent muscle columns tend to merge. This fact was well known to Kölliker (1867); 'it would be an error' he writes 'to conceive of these bundles as traversing regularly, side by side, the whole length of the muscle fibre, but on the contrary they are joined together and merge into one another.'

It remains to ask, what are the muscle columns and Cohnheim areas? Comparison of figures 18 and 19 shows that, allowing for slight shrinkage in the fixed preparation, the fibrils outnumber the areas by about 5 : 1; in some fibres the proportion is as little as 1·5 : 1. It would seem that the Cohnheim areas are cross-sections of bundles of fibrils, averaging about five per bundle, immersed in amorphous 'ground substance' ('Bindemittel' of Kölliker 1889), having similar optical properties to the fibrils, which are therefore not separately visible in the living tissue; and that adjacent areas are separated by darker interstitial substance, often containing granules. But the pattern of the interstitial reticulum varies along the length of the fibre, so that the muscle columns which it delimits merge at irregular intervals. It would seem, in short, that the peculiar Cohnheim pattern is an expression, not of a grouping of fibrils, but of the disposition of the interstitial substance. This does not imply that the form of the reticulum is without effect on the grouping of fibrils, and indeed close inspection of figure 19 seems to reveal minute groupings corresponding to the Cohnheim areas; but it is plainly not fibril groups that we see in a frozen section.

This interpretation of the Cohnheim areas, though quite at variance with most current statements, is fully in accord with the interpretation by Kölliker (1889) and Heidenhain

(1911). That it is the interstitial substance that determines the character of the Cohnheim areas and muscle columns we see at once in frozen sections of the flying muscles of birds. As is well known, these are very rich in interstitial substance. In cross-section they show an irregular network containing coarse refringent material, and with a wider and more irregular mesh than in mammalian muscle. This is shown in figure 25, plate 17, which represents a section of frozen unfixed pectoralis major muscle from a finch (*Zonaeginthus oculatus*). The corresponding section from a fixed and stained preparation is shown in figure 26, plate 17; observe the cross-sections of the fibrils grouped into irregular areas by the coarse-grained but weakly staining interstitial substance. In fibres of this type we should expect the columns of Kölliker to be much more pronounced than in mammalian muscle. Any longitudinal section at once confirms this (figure 27, plate 17); the photograph shows, at lower magnification, a muscle fibre with very well-defined streaks of interstitial substance, richly packed with interstitial granules. The muscle columns so delimited are of no constant thickness, and do not give evidence of their fibril content; observe particularly how adjacent muscle columns merge into one another, for the streaks of interstitial substance do not traverse even the short length of the fibre contained in the photograph.

In the present work our concern is with the muscle columns of arthropods, and in particular those of insects. To examine them the simplest and most effective method is that of frozen sections of the unfixed tissue, for here the interstitial reticulum is readily seen. It presents much variety of pattern in fibres of different sources. Usually the interstitial substance is of coarser texture than in vertebrates, and is commonly heavily charged with interstitial granules; accordingly, the effect upon the grouping of fibrils is more pronounced than is usual for vertebrate muscle. In most cases the reticulum is more irregular, and the meshwork wider, so that the muscle columns are of larger dimensions. But as with vertebrates the muscle columns do not usually preserve their individuality throughout the length of the fibre, for the pattern of the interstitial reticulum does not maintain any constant form along the fibre.

A few examples will serve for illustration. In the femoral springing muscle of *Chortoicetes terminifera* (plague locust) the muscle fibres are unusually thick—up to 150μ in diameter. Frozen sections show a coarse meshwork of granular interstitial substance, enclosing Cohnheim areas of irregular form and of unusually large size (figure 28, plate 17). In such fibres the muscle columns, as expected, are exceptionally well defined (figure 29, plate 17); observe the granular content of the interstitial substance that demarcates the muscle columns; observe also how, even within the small length of the photograph, adjacent muscle columns tend to merge into one another. Cross-sections of the fixed tissue (figure 30, plate 18) show an irregular grouping of fibrillar substance, corresponding to the Cohnheim areas of the frozen section. Another and unusual pattern is shown in figure 31, plate 18 (leg muscle of scarab larva *Aphodius howitti*); observe how in this case the granule-laden interstitial substance is disposed in irregular streaks that traverse the section, but with little tendency to enclose polygonal Cohnheim areas. The effect upon the grouping of fibrils is again evident in the fixed preparation (figure 33, plate 18). Still another Cohnheim pattern is shown in figure 36, plate 18 (leg muscle of cicada nymph). Here the interstitial substance forms a network that is rather more distinct than, but

otherwise similar to, that of mammalian muscle shown in figure 18, plate 17. In longitudinal section of the unfixed tissue (figure 35, plate 18) the muscle columns are well seen; the interstitial substance delimiting the muscle columns shows, in this instance, but few granules, while, on the other hand, there is a well-marked row of minute *J*-granules to either side of the *Z*-membrane, and not related to the interstitial substance between the muscle columns. For another illustration reference may be made to figure 44, plate 19, which shows a transected leg-muscle fibre from the prothorax of a grasshopper, *Caedicia olivacea*. Here the interstitial substance demarcates the cross-section into polygonal areas of irregular form and size, the pattern changing, around the margin of the section, into radiating lines, indicative of a lamellar structure of the fibrillar substance. Finally, it is to be noted that muscle fibres exist in which the interstitial substance does not form a reticulum of any kind, but is disposed in longitudinal streaks among the fibrils; an example, from the flexor mandibulae muscle of the cockroach, is shown in figure 41, plate 18.

In the types of fibre so far described, the interstitial reticulum does not preserve any constant form in successive cross-sections of the fibre, and therefore adjacent muscle columns do not keep their individuality throughout the fibre length. The well-known radial lamellar ('tubular') fibres are, on the other hand, often remarkable for the regularity of the pattern of their interstitial substance. These fibres form the leg or trunk muscles of many arthropods; figure 45, plate 19, is an example from the leg of the spider, *Pholcus littoralis*. We see here a pale nucleated axial column of cytoplasm, which radiates in thin pale lamellae to the margin of the fibre, demarcating the darker contractile substance into lamellae of about the same thickness. Owing to the transparency of the chitin, it is possible, even in the intact living animal, to make a close inspection of these fibres with an oil-immersion lens. In optical section along the fibre the lamellae then appear as parallel lines, easily mistaken for myofibrils (figure 46, plate 19); note that, for the whole length of the photograph, the lamellae preserve their individuality, without any tendency to merge. Since the lamellae are separated by interstitial substance, they conform to the definition of muscle columns. They constitute, however, a special case of another fibre component, distinct from muscle columns, yet not identical with myofibrils. This category of fibrils will be considered more fully now, and its relevance to the problem of the coarse fibrils of insect wing muscle will then become apparent.

(ii) *The fibrils*

The problem of the fibrillar construction of the muscle fibre is, for the histologist, an extremely difficult one. To what degree does the fixed preparation disclose the invisible texture of the living tissue? Is the limit of visible fibrillation in the fixed preparation set by the resolving power of the microscope, or are the finest visible fibrils the indivisible structural units of the fibre? And if this is so, then in what relation do they stand to the coarse fibrils of insect wing muscle, which are certainly present in the living tissue?

(*a*) *Existence of fibrils in the living muscle fibre.* The scepticism of some competent histologists on the existence of fibrils in the living tissue should not be regarded lightly. In cultures of embryonic heart muscle fibrils appear only after fixation, and have therefore been regarded as probable fixation artifacts (Lewis 1926). Or again, comparing the fixed

cross-section in figure 19, plate 17, with the frozen section of the same tissue (figure 18), it is plain that only the fixed tissue gives evidence of fibrils.

That the fibrillation in fixed material does express some form of pre-existing structure is generally conceded; what is disputed is whether the fibrils are present as such, or as some form of orientated micellae, that coagulate into fibrils at fixation; 'the most reasonable hypothesis is that mechanical tension produces lineal orientation of molecules in the living cell which on fixation we recognize as fibrillae' (Cowdry 1938). But the absence of fibrils may mean no more than that they are enveloped in a protoplasmic medium ('ground substance' of the fibre) with roughly similar optical properties. We need to recall that there are muscle fibres in which some form of longitudinal fibrillation is certainly present: coarsely fibrillated wing muscle is an example; the lamellated form of fibre is another (figure 46, plate 19). In small transparent arthropods, that often permit close inspection of muscle fibres *in situ* through the transparent cuticle, even with an immersion lens, a faint fibrillation is also often seen. Long ago Engelmann (1873 *a*) made a careful examination of such material, but saw the fibrils only when, through death or injury, they exuded a fluid into the interfibrillar spaces, which on fixation he recognized as 'ground substance' of the fibre. On the other hand, Schäfer (1873) and Wagener (1874) reported fibrils in undamaged fibres, while the remarkable photographs of Hürthle (1909) on living muscle fibres of *Hydrophilus*, with moving contractile waves, show them with a clarity approaching that of a fixed preparation. I have myself seen the fibrils under conditions where there could be no suspicion of injury to the tissue, the transparent animal being examined, with critical illumination, in a depression slide, in order to avoid pressure on the cover-glass. Figure 54, plate 19, shows an example from *Daphnia carinata*. Another example from the transparent leg of a scarab larva (*Aphodius howitti*) is shown in figure 32, plate 18; the optical conditions are here rather poor, for the chitin of the leg is curved and thick, yet the fibrillation is plainly visible, the double row of minute sarcosomes (*J*-granules) being also plainly seen between the *Q*-bands. A fixed preparation of *Aphodius* is shown, for comparison, in figure 34, plate 18. It is unnecessary to multiply examples; it is plain that fixation and differential staining accentuate the clarity of the fibrils, but there are no grounds for the belief that they are the product of such methods, even though they are not always visible before fixation.

(*b*) *Myofibrils and sarcostyles.* Heidenhain (1899, 1911, 1913), while fully recognizing the fibrillar texture of muscle, repeatedly stressed the difficulty of actually identifying the ultimate fibrils in the prepared tissue, single fibrils showing, with improved optical methods, a cleavage into yet finer fibrils, down to the limits of visibility. Does the cleavage proceed progressively into molecular dimensions (protomeres of Heidenhain), the fibril of the histologist being that aggregate of cleavage products beyond which optical resolution is not possible? Or does the fibril have a real existence? The improved resolution achieved with electron microscopy has decided in favour of the latter alternative, both for vertebrate muscle (Hall, Jakus & Schmitt 1946) and for arthropod muscle (Farrant & Mercer 1952), at least in the fixed tissue.

Heidenhain's observations raise a problem, however, that histologists have not properly recognized, but that the coarse fibrils of insect wing muscle bring to the fore: are the ultimate fibrils of which the muscular fibre is constructed, distributed within it as discrete

fibrils, or are they bound together into units of a higher order, but not identical with muscle columns? The existence of giant fibrils in insect wing muscle suggest the latter alternative as the more probable, for it is certainly surprising that muscles which are pre-eminent for the astonishing speed of their action, should have coarse fibrils; the reverse might have been expected, since the accessibility of the contractile fibril to molecules liberated in its proximity should decrease with increased thickening of the fibril.

In examining this question, it is necessary to stress the difficulty of presenting crucial objective evidence, since we are working at the limit of microscopical method. We turn first to the familiar 'radial lamellar' fibre, the strap-like fibrils of which have, since their first description by Aubert in 1853, been regarded as indivisible. Figure 45, plate 19, shows a cross-section, cut from the fresh unfixed fibre (the lamellae seem actually to be the pale lines between the darker lines of granule-laden sarcoplasm). My experience throughout the present work has been that such lamellae are most refractory to decomposition into subfibrils, and this conforms with orthodox opinion. Yet in one case Marcus (1921) has shown, with ultra-violet light, that the lamellae consist of a row of fibrils, embedded in amorphous ground substance. If in figure 112, plate 23, the reader will closely inspect the lamellae to the left of the nucleus, the same will be seen; and they are also readily visible in figure 55, plate 19. Even in cases where the lamellae appear otherwise homogeneous, the subfibrils can be seen at the insertion of the lamellae on to the chitin. This is shown, for instance, in figure 40, plate 18; the focus is so adjusted that on the right third of the fibre it is in the plane of a lamella, while to the left of this only transected lamellae are shown; note that the lamella, homogeneous in appearance, becomes resolved into separate fibrils shortly before its insertion, each fibril being connected, along the row of dots, with a tonofibril.

Consider, as another example, the fibre shown in figure 57, plate 20 (from the springing muscle of a fulgorid, *Scolypopa australis*). In the relaxed part of the fibre the fibrillation is very distinct, and we get the impression of giant fibrils, homogeneous in structure, that cannot be resolved into finer fibrils. Close examination shows that they are, in reality, bundles of most delicate fibrils, bound into units by ground substance. This is best seen at the insertion end of the fibre, where the delicate fibrils, each connected separately with a tonofibril, become fanned out a little, and thereby rendered more easily visible. This is shown in figure 60, plate 20, the photograph being taken in ultra-violet light to achieve maximum resolution.

I give, as a third example, the leg-muscle fibres of *Chortoicetes terminifera* (figure 30, plate 18). Here the fibrils are remarkably thick (1μ or more) and are grouped in accordance with the Cohnheim pattern, which in this muscle is unusually coarse (figure 28, plate 17). In figure 30 many of the fibrils do appear homogeneous; but many others give clear indication of composite fibrils that are held together by, and embedded in, some homogeneous ground substance. In comparing figures 30 and 28, note lower magnification in figure 28.

Owing to the importance of the subject for the present work, some additional examples will be given; in particular, we must be clear that the composite fibrils under discussion are not identical with Kölliker's muscle columns. In the leg muscle of the scarab larva *Aphodius howitti*, cross-sections of the fresh fibre disclose a peculiar interstitial pattern, but

in which the usual polygonal Cohnheim areas are not present (figure 31). In the living fibre viewed *in situ* through the transparent cuticle, a longitudinal fibrillation is visible (figure 32, plate 18); the darker longitudinal lines in this figure are actually not the fibrils, but the interstitial substance, the fibrils being the paler lines between the streaks of interstitial substance. In fixed preparations the interstitial substance disappears, but the fibrils survive and stain with remarkable clarity. This is shown in figure 34, plate 18; the photograph is at an enlargement of $\times 2000$, and close inspection can leave no doubt that the coarse fibrils have a subfibrillar structure. Comparison with the cross-section of the fresh fibre (figure 31) shows, moreover, that the coarse fibrils are not muscle columns, as defined above.

As a last example I take the large flexor mandibulae of the cockroach, a strong and incessantly functioning muscle, and therefore richly endowed with sarcosomes. A cross-section of the fresh fibre is shown in figure 41, plate 18; the coarse sarcosomes are very conspicuous, but a Cohnheim network is not visible. Muscle columns are therefore not present. If the tissue is frozen for a day or two, the sarcoplasm loosens, and the sarcosomes disappear. The fibrils then become evident; figure 42 shows them from a frozen section of the fresh tissue, seen with phase contrast. The fibrils are large, but give no evidence of subfibrillation. Are they, indeed, single fibrils, or have they a composite structure? The answer is given in figure 43, which is a cross-section, at the same magnification, of the fixed tissue; some of the smaller fibrils are possibly single, but the larger are plainly composed of two or three subfibrils, which in the unfixed tissue are obscured by the ground substance of the composite fibril. Are the fibrils muscle columns? The answer is surely in the negative, for they run a completely separate course along the fibre, and are not delimited by a Cohnheim net.

(*c*) *Discussion.* Sufficient evidence has now been given that the fibrils into which insect muscle fibres dissociate, and which are sometimes visible even in the living tissue, are not single fibrils, but are composed of several such, embedded in amorphous ground substance that binds them into a unit. Whether this is a general character of insect muscle we do not know; the examples have not been specially selected, and do not seem to be exceptional. In vertebrate muscle, where the fibrils are thinner, I have not been able to recognize composite fibrils.

In the extensive literature the existence of these compound fibrils has not gone unnoticed, both Kölliker (1889) and Retzius (1890) having described them; but they speak of them as muscle columns, without duly comparing them with the Cohnheim pattern. The above evidence shows that this is not the case; muscle columns are, indeed, merely an expression of the reticular character of the interstitial substance, the reticulum enclosing a variable number of fibrils into columns, which do not, however, retain their individuality along the fibre.

For convenience of description, and to emphasize their status, a name is needed for this category of fibril, and I propose that we adopt for the purpose the term 'sarcostyle' of Schäfer (1891), and that we confine it to fibrils of this order. It is descriptive and distinctive. It was intended to apply to muscle columns, but has, from the beginning, been used without distinction for muscle columns and fibrils, leading to much confusion in the literature. In the present paper it will be used strictly in the new sense here defined.

Definition. By a *sarcostyle* we mean a fibril, composed of subsidiary *myofibrils* of variable number, bound into a structural unit by amorphous *ground substance* in which they are embedded.

The term *myofibril* will be used in this paper for the fibrillar components of the sarcostyle; when a distinction between myofibril and sarcostyle is not in question, the simpler term *fibril* will suffice for either. The attachment of the myofibrils separately to the chitin by tonofibrillae emphasizes the belief that it is they, and not the ground substance, that are the contractile components of the sarcostyle, and it becomes a tempting hypothesis to localize within the ground substance of the sarcostyle the chemical breakdown that elicits contraction in the myofibrils. A peculiar feature connected with the cross-membranes of the sarcostyles, and which further emphasizes their unity, is referred to below (§D).

To avoid misunderstanding it should be emphasized that the subfibrillation of the sarcostyle into myofibrils is not the same as Heidenhain's view of a repeated cleavage of fibrils eventually into molecular dimensions. The myofibril is envisaged as the contractile unit, its indivisibility being shown by electron microscopy. What is important for the concept of the sarcostyle is the contractile myofibrils embedded in homogeneous ground substance, within which, it is supposed, the chemical breakdown that elicits contraction of the myofibril occurs. Indeed, even a single myofibril embedded in a film of ground substance would conform with the definition.

(*d*) *Status of the coarse fibrils of insect wing muscle.* The question whether these are very thick myofibrils, or sarcostyles as here defined, has physiological implications, and must influence our views regarding localization of events within the muscle fibre. Contraction is clearly the function of the myofibrils. If the chemical breakdown proceeds in the spaces between them, as seems not unlikely, then it is surprising to find enlarged myofibrils in a type of muscle that is distinguished beyond all others by its speed of action.

Many writers (Merkel 1872; Engelmann 1873*a*; Limbeck 1885; Kölliker 1888; Meigs 1908; MacDougall 1897; Jordan 1920*b*; Marcus 1921; Ciaccio 1940) look on the coarse fibrils as true myofibrils, and this is now generally accepted opinion. Opposed to this are Krause (1869), Ranvier (1880), Retzius (1890), Knoll (1891), Heidenhain (1911), Kielich (1918) and Janisch (1924), who regard them as composite structures, with the status of muscle columns. Schäfer (1891, 1912) refers to them as 'sarcostyles' (meaning muscle columns; see Schäfer 1891, p. 190), yet states explicitly that they are 'large fibrils' that might disclose important evidence in regard to the contractile process.

Those writers who regard the coarse fibrils as composite do so on the ground of their unusual size, and not by the demonstration of their component fibrils, which are believed to be of ultramicroscopic dimensions (Janisch, however, reports subfibrils in *Bombus* after swelling the fibrils in water). The opposed view, that the coarse fibrils are enlarged single fibrils, rests on the absence of visible subfibrils. Jordan (1921*b*), indeed, describes the presence of visible 'metafibrils' within some of the coarse fibrils, but accepts the view of Heidenhain of the divisibility of all myofibrils.

Opinion regarding the status of the coarse fibrils evidently still rests on conjecture rather than demonstration; and as important consequences, notably the localization of processes within the living fibre, are involved, a solution of the problem is much needed. Evidence will be given below that the coarse fibrils are not single enlarged myofibrils, nor yet muscle

columns, but that they are sarcostyles as above defined; but until the evidence for this is given, it will be best to refer to them merely as 'coarse fibrils'. The further question will then arise of discovering transitional forms between them and thin sarcostyles, and, if possible, of relating their exceptional structure to their special function.

C. *Sarcoplasm*

By this term we designate the cytoplasm within which the sarcostyles are embedded; common usage extends the term to the central axial column of nucleated protoplasm in the 'tubular' fibres (figure 56, plate 19), and even to the massive aggregation of protoplasm that we find, in some fibres, just below the sarcolemma.

If the chemical breakdown that activates the contractile mechanism of the myofibrils takes place within the sarcostyles, as seems not improbable, then the sarcoplasm is probably the locus of the various 'vegetative' activities within the fibre, including perhaps nutrition of the sarcostyles. Direct inspection of the sarcoplasm can contribute little to the understanding of these functions. The most that can be said is that it is plainly not a homogeneous substance, for apart from its content of sarcosomes, it displays in most fibres a darker 'interstitial substance' that ensheaths the muscle columns, and a paler 'ground substance' (Kölliker's Bindemittel) that directly bathes the sarcostyles. But neither in the lamellar type of fibre, nor in the coarsely fibrillated Siebold fibre of wing muscle, does this distinction seem to apply.

There seems no ground for disagreeing with orthodox opinion that the sarcoplasm, at least where it envelops the sarcostyles, has a viscous quality; this we infer from the absence of any Brownian movement, from its failure to percolate from a cut fibre (Heidenhain 1911), and from the fact that it seems to hold the sarcostyles together (Barer 1947). The same cannot be said of the axial column of sarcoplasm of 'tubular' fibres, the contents of which flow readily when a contractile wave passes along the fibre.

The sacrosomes are morphologically of two different kinds, but they need not be simultaneously present:

(*a*) J-*granules*

The existence of these was first made known by Retzius (1890). They are very minute, and are transversely alined in double rows, one to either side of the *Z*-membrane. Hürthle (1909) regarded them as artifacts, on the ground that they were not visible in the living fibre; yet in the uninjured living fibre shown in figure 32, plate 18, they are readily visible. Both in the fixed preparation (figure 37, plate 18), and sometimes even in unfixed material, they can be seen to be transversely connected into networks, which we identify with the 'sarcoplasmic reticula' referred to long ago by Rollet (1885). Hitherto the fact seems to have escaped notice that these minute sarcosomes are related to the sarcostyles, i.e. that they lie within the muscle columns, and not in the interstitial substance between them. This will at once be seen in figure 35, plate 18.

(*b*) *Interstitial granules*

These lie in the interstitial substance, i.e. between the muscle columns (figure 29, plate 17). They are larger than the *J*-granules, and are disposed in longitudinal rows, but in none of the muscles that I have examined, excluding wing muscle, are they also trans-

versely alined. Their disposition in figure 56, plate 19, merits special comment; this is an optical section along a living muscle fibre from a fly's leg, the fibres being of the radial lamellar type, in which, however, the lamellae are broken into two concentric rings by an intervening ring of sarcoplasm which is itself concentric with the axial core of sarcoplasm. The latter is the thick nucleated column that obtrudes in figure 56; the transected outer rim of sarcoplasm is seen to either side of it. Note the sarcosomes, both in the axial core and in the outer sarcoplasm ring, and their absence between the lamellae. In dragonfly wing muscle, on the other hand, Marcus (1921) has shown that the sarcosomes lie also between the lamellae; I can confirm this statement.

There are great differences in the content of interstitial sarcosomes in the fibres of different muscles, and this is plainly correlated with the activity of the muscle. The incessantly functioning mandibular muscle of the cockroach (figure 41, plate 18), springing muscle of grasshoppers (figure 29, plate 17), or flight muscles of insects, are rich in sarcosomes; in the sluggish leg muscle of cicada nymphs (figure 35, plate 18) or of scarab larvae (figure 32, plate 18) there is a dearth of interstitial sarcosomes.

Since the sarcosomes are not primarily associated with the fibrils, there is no reason to suppose that they play a direct role in contraction. This has, indeed, been advocated by Holmgren (1910) and Marcus (1921); but a location of sarcosomes as in figure 56, plate 19 is clearly inconsistent with this. Their true function lies evidently along different lines; recent work by Watanabe & Williams (1951) shows that they are loci of enzymatic activity, and that the cytochrome is contained within them.

In connexion with the sarcoplasm reference must be made to the 'trophospongium', which used to attract much attention, particularly in insect wing muscle, where it is the subject of a large memoir by Holmgren (1908). It is revealed by the same methods as were formerly used in routine studies on 'Golgi networks', i.e. osmic bichromate fixation followed by silver impregnation, and from this an identity between the two has been inferred. Holmgren, as is well known, envisages the Golgi network as an intracellular 'canalicular system', that itself arises by ingrowth of filaments from surrounding 'trophocytes', a nutritive function being claimed for the trophospongium, as indeed its name implies. That silver impregnation can, in wing muscle, reveal such an intracellular network has long been known (Cajal 1890; Veratti 1902); actually it constitutes, at least in part, the network of intracellular tracheae, as Holmgren indeed admits. But the method is said to reveal similar networks even in mammalian muscle, and from this Holmgren infers that the intracellular tracheal network and the trophospongium are 'completely identical'.

The structures shown in Holmgren's illustrations are very remarkable. In many cases they refer plainly to the network of hollow air-filled tracheae, and we merely confuse the subject by alluding to such a tracheal network as a 'trophospongium'. But the paradox of reported similar structures in mammalian muscle remains. In preparations of such muscle I have seen the structures that Holmgren describes—fine transversely running filaments beset with granules, and lying to either side of the *Z*-membranes, but I cannot see any connexion with surrounding blood vessels. They seem to be the transverse sarcoplasmic reticula above referred to, and cannot form the basis for a theory of a nutritive 'trophospongium'. There is, at present, no evidence for a muscle trophospongium, and we are not, therefore, impelled to seek it in insect wing muscle.

D. *The cross-membranes*

Here we are concerned with (i) the *Z*-membrane (Krause's membrane or telophragma) and (ii) the *M*-membrane, or mesophragma. The existence of the former has gained wide though not general assent; the *M*-membrane is still regarded with scepticism. Despite dissent in the literature, we cannot easily avoid the conviction that some such membranes must exist, for we are confronted with the fact of the transverse alinement of striations.

(i) *The Z-membrane*

As early as 1849 Dobie described the fine dark lines that bisect the clear bands between successive cross-striations. The lines of Dobie are among the best defined visible structures of the muscle fibre, and can be seen in the living tissue, or in frozen section of unfixed material (figures 20, 62*a*, plates 17 and 21). That Dobie's lines represent cross-membranes that transect the fibre seems first to have been recognized by Krause (1869), the earlier work of Amici (1859) being vague and uncertain. According to Krause, adjacent 'ground membranes' (i.e. *Z*-disks) of the fibrils are joined together to form a transverse membrane, being connected also at the margin to the sarcolemma, which, as Amici first saw, adopts in consequence the familiar appearance of 'festooning'. In the ensuing controversy about the reality of the cross-membranes, the presence of the *Z*-disks within the fibrils was never in dispute; they are indeed easily seen, even in living fibre (figure 54, plate 20), and it is to them that the dotted appearance, commonly presented by the cross-lines, is due. On the other hand, thin sections, even on the closest scrutiny, usually fail to show interfibrillar connexions between them. Schäfer (1873) and Hürthle (1909) deemed their presence to be incompatible with the aberrations in the cross-striation pattern, that are familiar to every histologist; but it is more likely that these aberrations are merely an expression of the helicoidal disposition of the striations (see below, §E). In the later writings of Schäfer (1891, 1912) the existence of these membranes is not recognized. Rollet (1885) attributed the optical and mechanical properties of the supposed membranes, including the peripheral attachment to the sarcolemma, to the 'transverse sarcoplasmic reticula', already referred to above (§C). Kölliker (1889), Retzius (1890) and Barer (1948) also deny their existence. On the other hand Flögel (1872), Engelmann (1873*b*) and Cajal (1888) affirm their presence. The decisive investigation was that of Heidenhain (1898, 1899, 1911), whose improvements in stain technology enabled him to display sharply stained cross-membranes completely transecting the interfibrillar spaces.

It is strange that fifty years after the work of Heidenhain the reality of the *Z*-membrane can still be doubted, at least in fixed tissue; in well-prepared mammalian muscle (fix in Apathy's alcohol corrosive-sublimate fluid, and stain with thiazine red and methylene blue, as recommended by Heidenhain) the *Z*-membranes can be consistently demonstrated, not merely in occasional fibres, but throughout the entire tissue. Figure 22, plate 17, is from material so prepared; figure 21, plate 17 shows the membrane from an orthodox haematoxylin preparation. For invertebrate muscle the latter method is more effective (figure 37, plate 18); the latter figure is of unusual interest, being from a type of fibre that possesses Rollet's 'transverse sarcoplasmic reticula' to either side of the *Z*-membrane. The distinction between *Z*-membrane and reticulum is evident, and the photograph shows that

the marginal connexion to the sarcolemma concerns the *Z*-membrane and not the reticulum, as claimed by Rollet. Exceptionally the *Z*-membrane can be demonstrated even in the fresh tissue: figure 98, plate 22, for example, shows it by phase contrast in a frozen section of fresh wing-muscle of a butterfly; figure 104, plate 22, shows it in the fresh fibre of *Blattella germanica*.

But while agreeing with Heidenhain that the *Z*-membrane transects the fibre, we may doubt its homogeneous character, which does not accord with the fact that it usually appears as a row of discrete dots (*Z*-disks of fibrils). The electron micrographs of Hall *et al.* (1946) show the *Z*-disks of the fibril as strictly confined to the fibril itself, without sign of marginal tearing, and this is also the common appearance with light microscopy. Actually, when the interfibrillar connexion is visible, it commonly appears fainter than the *Z*-disk of the fibrils (figure 37, plate 18), and von Boga (1937) finds the staining reactions of the two to be dissimilar. We need, therefore, to distinguish a fibrillar and an interfibrillar component of the *Z*-membrane, for which, though with a different connotation, the terms *Zf* (fibrillar) and *Zs* (sarcoplasmic) of Heidenhain may be used. Throughout the present paper I shall avoid the use of the terms 'telophragma' and 'Krause's membrane'; the terms *Zf* and *Zs* will be found convenient for descriptive purposes, *Z* alone being used when a distinction between its parts is not in question.

Does the *Z*-disk completely transect the sarcostyle, or is it only a product of its component myofibrils? It seems that the former is actually the case, and this again emphasizes the importance of the sarcostyle as a structural unity. In figure 61, plate 20, for example, which shows the sarcostyle of the springing muscle of *Scolypopa australis* (Homoptera) photographed in ultra-violet light at $\times 2140$, and in figure 34, plate 18, from *Aphodius howitti*, the *Zf* disks appear as thick bands completely transecting the sarcostyles, and not as alined rows of *Z*-disks of their component myofibrils. The coarse fibrils (i.e. giant sarcostyles) of insect wing-muscle, as will be shown below, afford a particularly clear instance of this.

Turning now to the flying muscles of insects, it is not surprising that in Orthoptera (Jordan 1919) and Odonata (Marcus 1921) *Zs* membranes have been described transecting the interfibrillar spaces, for these muscles closely resemble normal leg muscle. In the coarse fibrillar (Siebold) type of muscle the *Z*-disks of the thick fibrils are always easily seen, even in the living tissue (figure 63, plate 20), but the existence of interfibrillar connexions is uncertain. Neither Kölliker (1888) nor Schäfer (1891, 1912) admit them, and Thulin (1915) is emphatic that the flying muscles of the various species that he examined are 'grundmembranlos'. Morison (1928) could not see them in the bee, nor Poisson (1924) in various Hemiptera, but Jordan (1920*b*) and Ciaccio (1940) have described them in the wasp and beetle respectively. The same reason exists for inferring their presence in coarsely fibrillated wing muscle as elsewhere, the striations being transversely alined, though very easily disorganized in handling the tissue; and it will be shown below that in all muscles examined such membranes are, in fact, present.

(ii) *The M-membrane*

This remains one of the most elusive components of the muscle fibre. As early as 1868 Hensen spoke of a 'Mittelscheibe' transecting the fibre at the middle of the *Q*-band; unlike the *Q*-band, it is isotropic in polarized light (Engelmann 1873*b*) and can by this

means be made evident in the fresh fibre even when otherwise not visible; it is, for instance, faintly visible in the relaxed part of the fibre shown in figure 62*b*, plate 20. In the more recent literature the *M*-membrane is a membrane of the utmost delicacy, completely transecting the fibrils and interfibrillar spaces, midway between the *Z*-membranes, its principal sponsor being Heidenhain (1899, 1911). The *Mf*-component of such a membrane is often visible, and its presence is now confirmed by electron microscopy (Hall *et al.* 1946); it is the alined *Mf*-disks that probably constitute Hensen's line. But even with well-marked *Z*-membranes, complete *M*-membranes, showing *Ms* connexions, are usually not seen. Yet in proper preparations their presence can be demonstrated. Heidenhain's technique of mordanting with thiazine red after corrosive fixation, and staining with methylene blue is the best procedure; iron-haematoxylin is less effective. Figures 22, 23, plate 17, show the membrane prepared by the former method. As described by Heidenhain, *M* is a most delicate membrane, finer than *Z*, transecting the interfibrillar spaces and connected at the surface of the fibre with the sarcolemma (figure 24). Gaps in the membrane in figure 22 show that it is less resistant to stretching than is *Z*. It may be added that electron micrographs by Pease & Baker (1949) on thinly sectioned fibres also show the *M*-membrane.

Is the *M*-membrane an integral part of all striated muscle fibre? Important consequences are implied in this question, for the delicacy of the membrane and the precision of its arrangement point to some much more elusive function than that of merely binding the fibrils together. In vertebrate muscle it seems to be of general occurrence, for I have found it in random samples of vertebrates of various classes. But in arthropod muscle, where Jordan (1916) and von Boga (1937) have described it, I have myself only occasionally seen it, even with good staining of *Mf*-disks. Here is a case where the data of vertebrate muscle may not be adopted for arthropod muscle without further inquiry; and until an adequate technical method is found, the universal existence of an *M*-membrane must be left an open question.

Turning to the flying muscles, it is actually in the coarse Siebold fibrils that Merkel (1872) first saw the *M*-disk; but though many have confirmed this, an *Ms*-membrane has not been recorded. Surprisingly a complete *M*-membrane has appeared, in the present work, in wing muscle from a number of different species (description below).

E. *Organization of fibrils within the muscle fibre*

This is a subject to which histologists have hitherto given very little attention, yet it is likely to play a central part in any successful theory of the muscle fibre; the transverse alinement of the striations is indeed a very remarkable fact, since unalined striations would seem to serve equally well the mechanical function of the fibre.

It will be well to dispel at once the suggestion that the cross-alinement is no more than an expression of the property of the fibrils to multiply by longitudinal cleavage. When, as in *Salmo* (Heidenhain, 1913), the entire system of fibrils within a single fibre is the progeny of a single parent fibril, then the alinement is doubtless established in this way. But in the wing-muscle of many insects, as will be described below, it is, as it were, 'deliberately' imposed on the fibre, new fibrils being built into the growing fibre by a succession of separately incorporated myoblasts, in such a way as to establish the cross-striation.

It is hard to avoid the belief that this organization of the fibrils is at the base of the co-ordinated action that is seen in the familiar slow 'contractile waves' that appear in moribund muscle of some arthropods; figure 39, plate 18, for example, shows the contractile waves in a fixed preparation, and gives the impression that the contraction is passing along the fibres with a 'wave front' in the plane of the cross-striation, and not independently along the fibrils. To the objection that these are artificial contractions, due to fixatives, it will suffice to note that the instantaneous photographs of slow contractile waves, obtained by Hürthle (1909) in living muscle, show the same features. The transverse alinement is clearly bound up with the presence of the cross-membranes, and this points to the probability that these membranes are in some way involved in co-ordinating the action of contiguous fibrils. Those histologists who recognize the existence of the cross-membranes, usually ascribe to them some mechanical or supporting function; in particular, it is claimed that they maintain the cross-alinement (Heidenhain). But this does not meet the question of the need for maintaining such alinement.

The form of the contractile wave is presumably an indication of the path of propagation of excitation within the fibre. The current hypothesis is that a wave of depolarization traverses the sarcolemma and directly stimulates the underlying fibrils (through mediation of local chemical breakdown). Whether this can explain the orderly character of the contractile wave we may doubt. It might suffice for very thin fibres, but how can it apply to the giant wing-muscle fibres described below in Diptera? A 'wave front' of the type shown in figure 39 is also clearly inconsistent with propagation of the excitation diffusely through the substance of the fibre from the nerve-ending; it seems to indicate, rather, that the excitation had spread across the fibre before advancing along it. Propagation along the fibrils is excluded on similar grounds, and in any case, leaves unanswered the question as to how the excitation, in very thick fibres, spreads to fibrils not in close contact with the nerve terminal. It is, indeed, becoming increasingly probable that transmission of excitation within the muscle fibre is the direct function of the cross-membranes. There remains the difficulty of transmitting the excitation to successive cross-membranes. Possibly the sarcolemma, to which the cross-membranes are usually directly attached, is concerned; but there are also insistent claims that in most, but not all, muscle fibres, successive cross-membranes are joined to one another as parts of a helicoid, and this would give the conditions for a continuous transmitting system along the interior of the fibre.

This question of the helicoidal organization of the fibrils will now be considered in some detail, for it merits better understanding than is at present accorded it. The current view still is that the cross-bands (striations), as seen with the microscope, represent focal sections of a succession of transverse disks. This view stems ultimately from the well-known finding of William Bowman (1840) that the contents of the macerated fibre disrupt, on occasion, into discrete disks, rather than into longitudinal fibrils, and was part of his general and valid argument, in opposition to van Leeuwenhoek, that the striations are not marginal girths on the fibre, but that they are within the fibre. Bowman did not have to consider the possibility that his 'disks' might be fragments of a helicoid, since the question had not then arisen; nor would it have been an easy observation to make, for even in unmacerated fibres the disk pattern may be hard to distinguish from a closely wound helicoid. Since

'disruption into disks' has been repeatedly brought as an argument against the helicoid theory, it may be noted that Aurell & Wohlfart (1936) show photographically (see figure 26 of their paper) that the 'disks' are indeed fragments of a helicoid.

It must at once be said that in certain very thin fibres, such as those of the gut of insects, the striations are true disks, and this has never been questioned (Tiegs 1922*a*, 1934). The repeated statements in the literature of a spiral (i.e. helicoidal) disposition of the striae really apply to the ordinary thick skeletal muscle fibres, though even thin fibres of the extra-ocular muscles conform. The earliest statement on this subject is actually in a letter by van Leeuwenhoek to the Royal Society. The only reference accessible to me is in the *Collected Letters* (Delft edition) of 1718. The relevant passage, in the twelfth letter (translated for me by Dr J. Smit), reads: 'In my earlier letter I stated several times that the minute muscle fibres have circular girths, and that each ring-like girth is a circular band, in which, till now, I have been erring; for now I find it necessary to conclude that the girths of the fibres do not consist of rings, but are made in such a way as if we wound around a needle a very thin attenuated silver or copper wire; and that in winding we leave a little more space between the wire that is wound around the needle, than the silver wire itself is thick; or otherwise as the thread on a copper or iron screw goes.' After Bowman found that the striae were not marginal, this astonishing observation was forgotten.

Since Leeuwenhoek's day the helicoid ('spiral') has been repeatedly rediscovered with the aid of the modern microscope. Rouget (1866) and Daday (1895) have found it, and Münch gives a very satisfactory account of it. Heidenhain (1911) interpreted the puzzling 'vernier effect' as an expression of a 'screw structure', and in a later paper (1919) actually found the 'spiral', but regarded it only as an abnormality. I accidentally found it in 1922, and described it more carefully in 1934. D'Ancona (1929) and Aurell & Wohlfart (1936) now confirm its existence, but adverse criticism (discussed below) has discredited it.

A helicoid in longitudinal section can be distinguished from a disk pattern only when the section is axial. The appearance of such a section is discussed in a previous paper (Tiegs 1934): if the axis of the helicoid is in the plane of section, then a 'broken striation' effect is obtained, which becomes a zigzag line if the section is sufficiently thick; if, on the other hand, it is at a slight angle to the section, then we obtain Heidenhain's 'vernier' (the term, of course, lacks precision, but it will suffice).

In insect muscle, where the striae are often much wider apart than in vertebrate muscle, such appearances are readily seen; figure 52, plate 19, shows the vernier, figures 57 and 59, plate 20, the zigzag line. Figure 52 emphasizes the fact, referred to in my earlier paper, that a vernier may grade into a zigzag (note in the figure that in the middle of the vernier the *Q*-band actually shows the zigzag). Other examples of the vernier are shown in figures 23, 38, 58, 62*b*, obtruding less in those fibres in which the striae are close together. Finally, in the vertebrate fibre shown in figure 21 the photograph must be closely scanned to see it, and, when found, we are apt to dismiss it as a mere aberration.

Before discussing their generality, we may usefully examine the objections that have been brought against the interpretation of helicoidal striations. That muscle fibres are unusually prone to distortion needs no emphasis; in particular, uneven longitudinal tension may draw the cross-bands into oblique positions, bringing those on one side of

the fibre markedly out of alinement with those on the other. But this, in itself, cannot convert a discoid into a helicoid pattern, unless a group of fibrils is so displaced as to rupture a pre-existing disk; in fact, the question at issue is not whether the optical effects express a helicoid pattern, which they surely do, but whether this pattern has been artificially produced by fibril displacement. Jordan (1933) has objected that the effects are 'the resultant of shearing stresses or oblique tensions', and Woollard (1930) ascribes them 'to the dark appearance of the longitudinal bands. These shift in position as the focus is varied, and this is the explanation of the apparent continuity of Krause's membrane.' Would any histologist condone this criticism when applied to figures 57 or 59? The question of fibril displacement, which Speidel (1937), Feneis (1938) and Barer (1948) raise, needs more careful consideration:

(i) Consider figure 23, plate 17. This is a section along a rat muscle fibre, prepared to show the cross-membranes, both *Z* and *M* being visible. The helicoid effect is readily seen. It would not be possible to disrupt a disk pattern into such a helicoid without tearing the cross-membranes. The photograph shows actually that the cross-membranes are not torn. Similarly in figure 21, plate 17, the vernier is really a vernier of the *Z*-membrane.

(ii) In figure 57, plate 20, showing a helicoid in a fibre with fixed contractile wave, it is possible that fibril shift could explain the zigzag at one end, or at the other; but it could not explain both, because of inequality in the required amount of shift at the two ends. The helicoid must, in this case, have been present in the living fibre.

(iii) If a shift of fibrils is the cause of the vernier, then such a shift must be detectable in very short fibres, like those of very minute insects. This test is crucial. Consider figure 47, plate 19. This represents a section cut along three abdominal segments of a late nymph of *Thrips*, the whole insect measuring only 0·9 mm in length. It shows three successive fibres of the ventral longitudinal muscle, together with their intersegmental attachments (except the last). By good fortune the helicoid of each fibre has been axially sectioned, bringing the vernier into view. In places, despite the thin section, the full turn of the helicoid can be obtained by focus adjustment (figure 47*a* and 47*b*, representing a piece of the left fibre at higher magnification). If in these three fibres there has been a shift in fibrils, to produce a spurious vernier, in which direction has the shift taken place? And how has the 'distortion' produced the extra striation along the inner side of each fibre?

Plainly in at least some fibres the pattern of cross-striation is not discoidal but helicoidal; to what extent is it the normal pattern? In the ordinary fibres of vertebrate muscle, in my experience, the pattern is helicoidal. The fibres are here mostly thick, the striations very closely spaced, and the helicoid therefore more difficult to see. It is, of course, best seen in stained sections; but only a complete intact fibre can be used to exclude its presence. If we carefully focus with a high-power objective through the transparent fibre, the optical effects of focusing through a helicoid can, with some practice, be seen; for photographs both of stained sections and of optical sections along intact fibres, see Tiegs (1934). If the reader will compare these photographs with those given in the present paper, he will recognize their obvious identity, and be little inclined to dismiss them as mere artifacts. Against the validity of these findings Barer (1947) brings the surprising objection that there is no 'evidence of uncoiling of a spiral when the swollen muscle substance is allowed to flow through a spontaneous rupture in the sarcolemma'. The helicoid is surely a pattern

of organization imposed on the fibrils, and not an elastic spring; suppose the fibre shown in figure 57 to have ruptured its sarcolemma, should we expect it to uncoil through the tear?

Turning to arthropod muscle, we may consider first the 'tubular' type of fibre, i.e. with axial core of nucleated protoplasm, and usually with radial lamellar pattern of sarcostyles. These are best examined when the axial core is narrow. I select, as example, the leg-muscle fibres of *Tabanus imperfectus*. A cross-section is shown in figure 53*d*, plate 19; the sarcostyles are here disposed in rings around the nucleated core of axial cytoplasm. An axial section along the fibre is shown in figure 53*b*; figure 53*a* is focused just 'above' the axis (i.e. nearer the observer); figure 53*c* is 'below' the axis. At a hasty glance the helicoid is certainly not evident. Consider first the axial section (53*b*); if the reader will count the striations, he will find an excess of one along the upper half of the fibre, as indicated by the numbering (evidently the axis of the fibre is not perfectly in the place of focus)—this is the 'vernier effect' which disappears when the focus is taken above (53*a*) or below (53*c*) the axis. Using the nuclei as guide consider now any particular striation; the reader will see that the 'shift' that develops progressively from left to right in the axial section (53*b*) can now be obtained for any single striation by focusing down from the plane of 53*a* through the plane of 53*b*, to the lower plane (53*c*)—to facilitate observation I have numbered a select striation (number 10–11). The pattern is clearly helicoidal. All the leg-muscle fibres of this species are of this type.

A comprehensive examination of arthropod muscle, with a view to disclosing exceptions, is beyond the scope of this paper. Speidel (1939) finds that in a pycnogonid the fibres are without helicoidal pattern. I can confirm this on a local species. In the leg muscles of a housefly that I have examined for the purpose, I find that an occasional fibre, always rather thin, is without helicoid pattern. It will eventually be necessary to inquire into the meaning of these exceptions; for the moment we can but express the conviction that a helicoid pattern, in organisms so dissimilar as vertebrates and *Thrips*, is not likely to be fortuitous. The reader who is still inclined to regard the helicoid as an infrequent exception should examine published illustrations of muscle, in which photographs are used in preference to drawings (e.g. Hürthle's paper, 1909); he will be surprised at the frequency of the 'exceptions'.

I have used the abdominal muscles of *Thrips* for some observations on the development of the helicoid. In the minute first-instar nymph the fibres are very short, each with two cross-striations only, and with a single intervening *Z*-disk, and the helicoid is not present (figure 48, plate 19). This is succeeded by a 3-striation stage, still without helicoid (figure 49, plate 19). At the 6-striation stage a helicoid is plainly present (figure 51, plate 19). How has it arisen? If we examine figures 50*a*, *b* we see two fibres at a stage intermediate between 3- and 5-striations, a new striation being in process of formation at the ends of each fibre. The fibres have themselves undergone thickening, presumably by fibril increase, but the former disk pattern is giving way to a helicoidal pattern, for the cross-bands of certain of the new fibrils have come to lie midway between the cross-bands of the older fibrils (in the case of one of the fibres, at least four new fibrils are involved, in the other, shown at adjusted focus in figure 50*b*, seemingly only two fibrils are out of position). I have not been able to make the much-needed observation on the behaviour of the

Z-membrane; it may well be that a change in configuration of the *Z*-membrane in the thickening fibre initiates the change in pattern of cross-striation.

I have referred, in another paper (1934), to various geometrical forms of the cross-striation pattern that may be included under the term 'helicoidal'. Some further reference to this is now called for. The pattern in figure 53, plate 19, is that of a helicoid wound about an axial cylinder; in fibres of this type the axial sarcoplasm is often thick, and the helicoid then difficult to see. A limiting case is given when the cylinder is reduced to zero, giving a helicoid wound about an imaginary line. Such a helicoid is expressed in longitudinal section as a vernier running parallel with the fibre axis (figure 23, plate 17). Much more abundant is a configuration in which the helicoid effect is obtained by focusing through a considerable depth of fibre (oblique vernier); the interpretation, given in my earlier paper, is that of a helicoid wound about a surface. Not at all uncommon is a double helicoid pattern. The existence of these has been denied even by recent supporters of the helicoid theory; the reader is therefore invited to examine figure 58, plate 20 (fibre on right), and to offer an alternative interpretation* (note incidentally the vernier in the adjacent fibre). Finally,† there are cases in which more than one vernier can be obtained from a single fibre; figure 23 shows a case where the two are simultaneously in focus. We are here evidently concerned with two helicoidal systems in a single fibre. The one thing that these diverse patterns have in common is that they bring about a continuity of successive cross-membranes.

Regarding wing muscle I have had no difficulty in detecting helicoidal structures at least in favourable cases; for coarsely fibrillated (Siebold) muscle, where information is much needed, observations are most difficult to make, owing to the tendency of the fibres to disrupt into fibrils when handled.

F. *Structure of the myofibril*

Here the most remarkable feature is the periodic transection of the fibrils by the *Zf*-disks into a succession of similar segments which, following Schäfer (1891), we will refer to as sarcomeres. If we envisage these as the contractile units of the fibre, then the hypothesis of their organization by the cross-membranes conveying the excitation presents at least an intelligible picture of the architecture of the fibre.

While electron microscopy of the fixed sarcomere has come to supersede its study by light microscopy, there are still a few points arising from the latter technique that merit comment, especially in their application to wing muscle. These concern the *Q*-band, and its behaviour during contraction. The *Q*-band is the dark component of the sarcomere, usually visible in the fresh fibre, where it occupies, at full relaxation, about the middle two-thirds, or even more, of the sarcomere. In the fixed preparation it shows a strong affinity for certain stains (e.g. haematoxylin), and both in the fixed and unfixed fibre is

* It will be understood that there is no intersection of cross-striations, as the photograph suggests; the two sets of striations are on two slightly different planes, but owing to their exceptional clarity appear within the same focal depth.

† It would be well to inquire whether the 'sphenoids' described at length by Heidenhain (1919) and Aurell & Wohlfart (1936) may not arise from fibre distortion. If the striae on the upper and lower half of a fibre are drawn strongly out of alinement by appropriate tension, a section along the fibre would display these effects if it traversed a slight wave in the fibre.

anisotropic in polarized light. When the fibre is contracted, the *Q*-band is no longer seen, either in the fresh tissue or in the stained preparation; there is, instead, a pronounced darkening and thickening of the transverse line in the position formerly occupied by the *Z*-band alone ('contraction band' of some authors). In this 'reversal of striation' the anisotropic substance does not participate, and is therefore presumably not identical with the dye-absorbing substance, which at relaxation forms the dark *Q*-band.

For the intact and unfixed fibre this is most readily seen in the 'stationary contractile waves' that appear in the moribund tissue of many arthropods. Figure 62*a*, plate 20, shows with phase contrast such a wave in a muscle fibre of the housefly; figure 62*b* represents the same fibre in polarized light with polarizing filter. Note in figure 62*a* how the fine dark *Z*-band of the relaxed fibre gradually thickens in the contracted zone, with an attendant disappearance of the intervening *Q*-band; note also how with polarized light the anisotropic zone remains fixed. I have chosen fly-muscle for describing these appearances, because here the picture is not confused by cross-alined sarcosomes (it will be recalled (see figure 56, plate 19) that the visible sarcosomes lie in an axial column, and in an outer ring concentric with this).

'Striation reversal' affecting the whole fibre was first described by Flögel in 1872; Engelmann (1873*b*), Rollet (1885) and Schäfer (1891) then confirmed it. That the reversal did not affect the anisotropic zone was first reported by Engelmann (1873*b*). The discordant statements in the literature regarding the interpretation of reversal arise mainly from the apparent discrepancy between the appearance in polarized and unpolarized light. Flögel (1872) and Merkel (1872) contend that striation reversal is a property of the fibrils, and this view has later been upheld by Rollet (1885), W. Rutherford (1897) and Jordan (1920*b*). On the other hand, Schäfer (1891, 1912), Retzius (1890), Holmgren (1907) and Heidenhain (1911) attribute it to a movement of interfibrillar sarcoplasm that is pressed against the *Z*-membranes.

In the case of fly muscle, the absence of interfibrillar sarcosomes at once excludes the latter interpretation. Stained and fixed sections can also leave no doubt that the reversal is, as Flögel and Merkel first claimed, a property of the fibrils. For example, in figure 38, plate 18, the 'contraction band' is plainly a darkening of the fibrils, the *Q*-band of the relaxed fibre being no longer in evidence (the point of attachment of the *Z*-membrane to sarcolemma is a useful guide). The effect is even better seen in the 'stationary contractile waves'; in figure 39, plate 18, for instance, there can be no question of any dark sarcoplasm accumulating against the *Z*-membrane, to produce a spurious reversal (see also figure 62*a*, plate 20). It is noteworthy that the electron micrographs of Hall *et al.* (1946) and of Draper & Hodge (1949) show reversal in the fibrils. The so-called *N*-band, occasionally seen in fixed material, seems to arise as an early phase in reversal (see Merkel 1881).

Before leaving this question, reference may be made to another source of confusion in interpreting reversal. In the fresh fibre the *Q*-band is often exceedingly faint, and the appearance of cross-striation is actually given by the *Z*-band. In such fibres we do not see reversal, and this explains Hürthle's failure to find reversal in *Hydrophilus*. The difficulty is accentuated when a prominent row of *J*-sarcosomes emphasizes the *Z*-band; for example, in figure 35, plate 18, the barely visible *Q*-band is overshadowed by the row of

J-granules. Similarly in figure 32, plate 18, it is the *J*-granules and not the *Q*-band that obtrudes, though in the fixed fibre the *Q*-band is strongly in evidence (figure 34, plate 18).

Striation reversal is the one fact that light microscopy can contribute to the problem of contraction, yet it does not play any part, direct or incidental, in current hypotheses of contraction. It seems that movement of a fluid component of the sarcomere out of the anisotropic framework into the isotropic zone is involved, and that a full understanding of this is likely to disclose the secret of contraction.

G. *Tracheal supply*

In leg or trunk muscle of insects the tracheae usually lie outside the muscle fibres, which, however, they closely invest; in exceptional cases they penetrate in small numbers into the fibres, especially if these are thick (e.g. trunk muscles of lepidopterous larvae). In remarkable contrast is the rich intracellular tracheation of wing muscle.

As early as 1828 Straus-Durckheim found, in *Melolontha*, the dense tracheal supply of the wing musculature. To Leydig (1859) belongs the credit of first showing that fine air-filled branches penetrate into the muscle mass, and ramify among the coarse fibrils. The presence of these air-filled interfibrillar tracheae has often been disputed (Ranvier 1880; Ciaccio 1887; van Gehuchten 1886; Münch 1903), but is confirmed by Limbeck 1885; Kölliker 1888; Schäfer 1891; Kielich 1918; and von Ebner 1918 and Wigglesworth 1950. For coarse fibrillar muscle there should be no difference of opinion on at least this point, for when fibres from a freshly killed insect are examined in glycerine with dark background illumination, the elaborate system of branching air-filled tubes is at once seen (figure 295, plate 32). On the other hand, in the very active flying muscles of dragon-flies, intracellular tracheae seem to be absent (Marcus 1921).

Only selective staining methods can bring out the finer details of the intracellular system. That the tracheae could be impregnated by the Golgi process was first reported by Cajal (1890), and applied to a variety of insects by Holmgren (1908). In coarse fibrillar muscle there is revealed, by this method, what appears as a system of most delicate transverse filaments that encircle the fibrils at the level of each *Q*-band, and are interconnected by fine longitudinal filaments. This is the 'trophospongium' of Holmgren, referred to above; but he acknowledges its tracheal origin in this particular case.

It cannot be said of the Golgi preparations that they have solved the question of the intracellular tracheae. That the tracheae can be displayed by this technique is certainly so; but it is disconcerting to find that similar networks have been reported by this method in crustacean and even mammalian heart muscle (Holmgren 1908).

By the use of Cajal's reduced silver method Athanasiu & Dragoiu (1913) have selectively impregnated the intracellular tracheae in *Hydrophilus* wing-muscle; they find a system of tubules less elaborate than figured by Holmgren, but corresponding closer to that seen in the living tissue, the tracheae running mainly lengthwise between the fibrils, but with cross-connexions that are said to be attached to the fibrils at the level of Hensen's line.

In the present work a more effective method, that of Da Fano, has been extensively used; with its aid not only the fully formed tracheae, but even their development can be followed. It has, however, the usual fault of all silver methods of being capricious in action, and in some species even fails entirely (e.g. mosquito).

H. *Innervation*

In the ordinary muscle fibres the nerves terminate by the well-known 'endings of Doyère', of which, it is said, there are sometimes many on a single muscle fibre. The 'nerves' are surprisingly thick, and consist of a nucleated sheath, itself derived from the sheath that invests the ganglion, and is continuous at its distal end with the sarcolemma of the muscle fibre. This sheath ('neurilemma') encases the true nerve fibres, axon out-growths from nerve cells that lie within the ventral ganglia. These are extremely fine and evade most staining techniques, and are present either singly or in numbers within the neurilemmal tubes. They end within the nucleated protoplasm of the Doyère end-organ below the sarcolemma of the muscle fibre, and there often undergo considerable branching. Mangold (1905) contends that in arthropods there are always two separate axons ending in the end-organ; Orlov (1924), however, finds in the visceral muscle fibres only one such axon. Until the question has been much more thoroughly investigated, no general statement regarding double innervation in insect muscle is possible.

For insect wing muscle Ciaccio (1887) described, in *Sphinx convolvuli*, a typical Doyère ending, while Mangold (1905), using methylene blue, finds in *Decticus* (Orthoptera) an elaborately branching system of nerves that end by double endings within the Doyère end-organs. For the coarse fibrillar muscles Arndt (1873) reported Doyère endings, several in number, in *Rhagium inquisitor* (Coleoptera). Cajal (1890), however, could find no trace of such endings; from Golgi preparations he described, in muscid flies and *Hydrophilus*, a system of large multipolar ganglion cells that branched and intertwined on the surface of the muscle fibres. This, if fully proved, would create for wing muscle a unique position among the striated muscle tissues. Cajal's claim has, however, been confirmed by Rina Monti (1893–4), and with some hesitation by Holmgren (1908), and is approved in modern standard works on insect morphology.

Here we see yet another of the reported anomalies presented by wing muscle that render difficult a comparison with normal muscle. In the present work this system of intramuscular ganglion cells has not been found. It will be shown below that silver impregnation can reveal the motor innervation of the wing muscles, and that, at least in some cases, the endings are even of the Doyère type.

Whether there are sensory nerves in the wing musculature is unknown; and certainly in the present work none have been found. Orlov (1924) seems to have found sensory nerves in insect visceral muscle, but for the trunk musculature these are unknown.

I. *Development*

Out of the prolonged controversy regarding the histogenesis of muscular tissue there has emerged a fairly general belief in the truth of Remak's doctrine that the multinucleate muscle fibre is ultimately a derivative of a single myoblast, or a very few such cells. The evidence comes mainly from a study of vertebrate muscle, and little credence has been given to the statement of Weismann (1862) that the muscle fibres of arthropods differ from those of vertebrates by their multicellular origin. From a comparison of vertebrate and crustacean muscle, Franz (1915) has shown the essential correctness of Weismann's observations.

Concerning the histogenesis of normal insect muscle, there is still much to learn. The most reliable observations relate to the muscles of the imago that develop in the pupa; but these are exceptional muscles, for their development is not attended by subsequent growth. They arise by fusion of a number of myoblasts into a column, either by replacement of larval muscles, or independently of such, the protoplasm then differentiating into a column of fibrils within which cross-striations then appear (Weismann 1862; Perez 1910; Poyarkoff 1910; Tiegs 1922*b*; Oertel 1930). Regarding the flying muscles we have very little exact information. The few available accounts point to a form of myogenesis wholly unlike that of any other kind of muscle hitherto described; the observations have, however, appeared mainly as incidental studies in memoirs on insect metamorphosis, and have not found their way into the general literature on muscle histology. As late as 1888 Kölliker, on the basis of van Rees's (1888) observations, still advocated the derivation of the fibre, even of wing muscle, from single myoblasts, though Weismann (1862) had described their development out of vast swarms of such cells. In *Calliphora*, according to van Rees, thousands of mesodermal cells cluster around certain persisting larval muscles; but he regarded these as the forerunners of nerves and tracheae, the adult muscles regenerating out of the larval muscles. The improved observations of Perez (1910) show, on the contrary, that the swarming mesodermal cells of van Rees are myoblasts that penetrate into the interior of the degenerating larval muscle fibres. We may doubt the correctness of Perez's description of the later stages of development, namely, that the larval muscle fibre, with its invading myoblasts, now forms a fused mass, out of which the myofibrils differentiate; his figures are, however, sufficiently remarkable to indicate a form of myogenesis radically different from that of any other known type. In the chalcid wasp *Nasonia* (*Mormoniella*), the swarming myoblasts co-operate in a strange way to form the new muscle fibre; they become progressively built into the enlarging fibre, each elongating and apparently generating a single coarse fibril in the process (Tiegs 1922*b*). In the Hemiptera, Poisson (1924) also has shown that myoblasts become progressively added to the growing fibre, though how this is achieved is not described. In the only investigation directed specifically to the development of the flying muscles, that of Jordan (1920*a*) on *Vespa*, a completely different account is given; an unspecified, but apparently small number of spindly myoblasts, whose nuclei multiply only by amitosis, is said to fuse into a column, and within this syncytial column, with its axial row of nuclei, the fibrils then appear, as in leg muscle. No account is taken of earlier work on the subject, and all the truly remarkable features of the process have been wholly overlooked.

The elucidation of this strange form of myogenesis was the initial object of the present work. If we adopt the still widely held view that development gives the clue to homology, then we are left with the uneasy result that there can be no homology between wing muscle and the other muscles of the insect body. The present work does not, however, support such a conclusion. It will show, on the contrary, that in the lower insects there are diverse types of histogenesis in the wing muscles; and though the data are still far from sufficient, they indicate an intelligible gradation towards the form of development that takes place in ordinary muscle during the growth of the nymph.

Turning to the finer details of the histogenesis of wing muscle—the source and production of fibrils—we find an almost complete absence of information; and even for normal muscle,

where formation of fibrils has been much studied, the accounts are discordant. Heidenhain (1911), on the 'protomere hypothesis' already referred to, envisages in the cytoplasm, even of the egg, the existence of self-propagating contractile particles; in the development of the specialized contractile tissues 'the smallest living particles aggregate into "molecular fibrils", and these by growth and longitudinal fission become grouped into bundles, which thereby attain the thickness of the microscopically visible'. In support of this he finds that in developing heart-muscle the fibrils do, indeed, seem to emerge as just perceptible filaments 'from the realm of the invisible'. These speculations, which savour of preformation, are opposed by claims that the fibrils arise from protoplasmic material that cannot be the equivalent of 'protomeres'. But concerning the character of this material there is much difference of opinion: Moroff (1912) derives it from disruption of nuclei; Benda (1899), Meves (1907), Duesberg (1910), Schaxel (1912), Luna (1913) and others consider it to be of mitochondrial character. Yet Cowdry (1918) is justly critical of all this work, for the chemical difference between mitochondria and myofibrils cannot be ignored. Godlewsky (1902), McGill (1919) and Häggqvist (1920) derive the fibrils from cytoplasmic granules of unknown origin; Weed (1936) believes that, in addition to such granules, mitochondria also contribute material. Finally, Gaudissart (1919) states that the mitochondria merely lie alongside the fibrils that arise from an unstained cytoplasmic reticulum. It will be shown below that in insect wing muscle the fibrils, except when they develop by cleavage of pre-existing fibrils, arise by coalescence of cytoplasmic granules within the myoblasts; but these granules do not seem to be mitochondria, for they survive the use of fixatives that dissolve these bodies.

On the question of the further development and structural differentiation of the fibril, little can be learnt from light microscopy, beyond the fact that the fibrils, at least in some muscles, are capable of longitudinal cleavage. The most impressive demonstration of this comes from Heidenhain's work on *Salmo* (1913), where the entire fibrillar content of a fibre is shown to arise from repeated cleavage of an originally single fibril. The few recorded observations on myogenesis in invertebrates give no example of this, nor do there seem to be any known cases of cleavage of entire fibres comparable with that described in some vertebrates (Maurer 1906). In the present work, however, ample evidence of the existence of such processes in insect muscle has been discovered, and will be described in detail below.

Regarding the development of new cross-striations as the fibre lengthens, Heidenhain (1911) finds that these appear only at the ends of the growing fibres. The example from *Thrips*, given above, confirms this. Throughout the present work this terminal formation of new striations has been found to hold, and not their intercalation along the fibre, and needs no further reference in the forthcoming description.

Histological methods employed

Examination of the fresh muscle fibre is not always as difficult as first acquaintance with the tissue suggests, and certain points of structural detail can be made out even more effectively on the fresh tissue than by any known method of fixation. This does not, however, apply to the fibrillar component, which usually requires the aid of fixation technique.

The question of artifact does not seem to obtrude seriously in the present work, except to the extent that fixation emphasizes or suppresses individual cell components. Where needed, comparison with the fresh tissue is made, and on the whole this has confirmed the general picture given by fixed tissue. Here only routine technique will be given.

Fibrillar fixatives

In my experience only alcohol-rich fixatives suffice for good fibril fixation: (i) *Carnoy's fixative*, a good general fixative for fibrils, at times gives even exquisite fixation; (ii) *alcoholic Bouin mixture*, though erratic, often gives superb fixation; (iii) *alcohol, acetic acid, trichloracetic acid* (*A.A.T.*), an adaptation of Heidenhain's trichloracetic technique. I have found the following formula of the greatest value: alcohol 95% (6 parts), glacial acetic acid (1 part), trichloracetic acid crystals (1 part by volume); make up freshly before use, and fix for about 6 to 12 hours.

There is, unfortunately, no single reliable general fixative, though a particular species will usually respond to at least one of the above. Other standard fixatives, such as Carl's fluid or the ordinary Bouin formula, do not give adequate fixation of fibrils.

Sarcosomes

Owing to their strongly refractile quality, these are still best examined in the fresh tissue. If this is impracticable fixation by osmic-bichromate mixtures (e.g. Champy's fluid) may be done; penetration is, however, slow, and serious artifact, particularly fusion of sarcosomes, is apt to occur. Formol alcohol is sometimes useful.

Cross-membranes

The lack of a standard technique to bring out these membranes is a serious hindrance to progress (Heidenhain's methods are applicable only to vertebrate muscle); in the present work I have had to rely on the chance preparation made by fibrillar fixatives. *Z* causes on the whole little trouble; *M* appears only seldom.

As a routine stain for fibrils, sarcosomes and cross-membranes, I have, in most cases, used Heidenhain's iron-alum haematoxylin. Sections were, in all cases, cut from wax-imbedded material.

Nerves

Although gold chloride is occasionally effective in insect wing muscle, the best preparations have come from silver impregnations. I have not had any success with the Bielschowsky or Cajal methods; the Da Fano process, on the other hand, often gives tolerably good nerve impregnation. A more effective method is that of Willis, which can now be used after Carnoy fixation. The original Willis process (Willis 1945) has been much modified, but the improved method has not yet been published. My preparations have all been made for me by Mr Willis himself.

Tracheae

Much the most effective method is the examination of fresh tissue, particularly after immersion in glycerine; figure 126, plate 23, will show the quality of the picture that this simple method will give. When this is not practicable, and especially in tracheae not yet

filled with air, silver impregnation must be resorted to. Cajal (1890) first described the use of Golgi impregnations for this purpose, and tracheae often impregnate embarrassingly in silver impregnations designed to bring out intramuscular nerves. The Da Fano method is the most effective for tracheal impregnation; yet some species are wholly refractory even to this.

Illustrations

To make the evidence objective I have used mainly photographic illustrations. The photographs are the work of Mr E. Matthaei, of the University Optical Laboratory. The following note is by him.

Optical methods (By E. Matthaei)

The Köhler system of illumination with carbon arc, or high-pressure mercury arc, was used, and with appropriate filters to give the best contrast. The routine high-power immersion work was done with a high-aperture immersion condenser, and Zeiss apochromatic objective (N.A. 1·3). This technique permits exploitation of the microscope's resolving power to its limits, and has sufficed for most of the work.

Special cases have required the use of special methods, as follows:

Phase contrast, with Zeiss achromatic objective, and a fixed phase shift of one-quarter wave-length.

Ultra-violet light photomicrographs, using a Bausch and Lomb monochromatic objective corrected for 365 mμ, and with a potential resolving power of 140 mμ.

Polarized light, using polarizing filters in preference to Nicol prisms, because they permit full exploitation of the condenser aperture. Muscle fibres were, in all cases, orientated at 45° to axis of vibration of the polarizer. Magnifications have been chosen with regard to the high demands of resolving power. In some instances they exceed the conventional value of N.A. $\times$ 1000, because of the likely loss of differentiation conditioned by (*a*) the photographic process, and (*b*) the printing process. In a few instances magnifications of the order of $\times$ 3000 have been used; some of these are justified by the use of ultra-violet light, while others are mere enlargements of negatives taken at conventional magnifications.

General histology of the wing-muscle fibres

Before passing to the wing musculature of selected orders of insects, it will much clarify the subject if we treat, collectively, certain of their general characters—the fibrils, sarcoplasm, cross-membranes and sarcolemma.

A. *The fibrils*

Here we are confronted with the problem of the diversity in the type of fibril among different insects, and its functional meaning.

It will be useful, at the outset, to emphasize the remarkable, and indeed unique physiological properties of the flying muscles, at least in the higher orders of insects. As is well known the 'indirect' muscles, by deforming the tergal thoracic wall, act by a lever mechanism on the wing: the dorso-ventral muscles by flattening the scutum, depress the wing base, and so cause the upstroke of the wing; the downstroke is due to contraction of the longitudinal muscles, which reverse the effect on the scutum. Strength can be imparted

to the wing beat if there is a long distance between the wing articulation (fulcrum) and the point at which the force of the scutum is applied, the gain in mechanical advantage being to the detriment of the speed of wing movement; accordingly, in large-winged moths and butterflies, where wing movement is slow, the thoracic wall is very flexible, and undergoes violent deformation in flight. If, on the other hand, the distance to the fulcrum is small, then the condition for rapid wing vibration has arisen; but whether rapid or slow, a short wing base must imply a minimum of movement of the thoracic scutum, so that the muscle contracts under conditions that are approaching isometric. Inspection of the thorax of a fly or a bee, with the wings rapidly vibrating, reveals, in contrast to that of the butterfly, an only just perceptible trembling, the thorax of many wasps being hard and unyielding. Low-frequency contraction under almost isometric conditions need not exclude the possibility of propagated shortening along the muscle; but high-frequency contraction, which is said in the mosquito to reach 500/s, and in the midge *Forcipomyia* to 1000/s (Sotavalta 1947), implies an almost instantaneous contraction of the entire muscle, and with a minimum of shortening.

But while isometric conditions facilitate high-frequency wing movement, it is evident that the muscle itself displays unique properties: the rapidity of the mechanical response in the wing muscles of many higher insects, and the absence of fusion of contractions even at these frequencies are highly remarkable; the muscle, moreover, is said not to contract in response to faradic stimulation (Kühne 1862), and it is hard to escape the belief that these two unusual properties are interdependent.

With some notable exceptions that will be considered later, it is especially in the rapid type of muscle that we find the coarse (Siebold) fibril. For reasons that have already been given (Introduction, p. 231) we should expect in these muscles, not thick fibrils, but the reverse, and we are therefore led to suspect that the coarse fibrils are not giant myofibrils, as generally believed, but that they are sarcostyles as above defined. Yet the photographs of Meigs (1908) of living fibrils in ultra-violet light give no hint of any subfibrillar structure, nor is it recognizable in the photographs of fixed fibrils by McDougall (1897), Holmgren (1908, 1910) and Marcus (1921), while Heidenhain (1911), convinced of its existence, relegated it to the ultra-microscopic.

It has come therefore as a surprise that the expected composite structure of the coarse fibrils can be readily and consistently displayed in histologically favourable material, the component fibrils falling well within the range of visibility of the light microscope. We begin with an examination of the coarse fibrils of the bee; the fibrillation of some members of the lower orders will then be examined, in the expectation that this will reveal an intelligible gradation, both structural and functional, between the highly specialized Siebold fibril, and the simpler fibril of more primitive insects.

(i) *The honey-bee and related species*

In these insects the wing-muscles, when exposed by bisecting the thorax, seem to remain unresponsive to faradic stimulation. The frequency of wing beat in the bee is given as 208 to 277/s (Sotavalta 1947).

In the honey-bee the coarse fibrils range in thickness from 2·4 to 3·4μ (average 3·2μ), the inter-Z distance being about 2·7μ, i.e. only a little less than the average thickness of

the fibril. (The inter-*Z* distance is most reliably measured not in the fresh tissue, but in muscle fixed without detachment from the chitin; the thickness is measured in fresh fibrils whose inter-*Z* distance is the same as that of fibres fixed *in situ*.) The fibrils traverse the entire length of the muscle, and are completely invested by sarcoplasm that separates them from one another (figure 63, plate 20).

Their appearance in the fresh muscle fibre is shown in figure 63, the photograph representing a group of fibrils, viewed intact through the transparent sarcolemma with phase contrast. The *Z*-membranes of the fibrils are sharply defined, while midway between them is a rather poorly defined *M*-membrane. Apart from this no structural detail is apparent by this method of examination.

When the fresh fibrils are isolated from the fibre they do not retract, but retain their normal length (figure 66, plate 20); the improved optical conditions do not, however, reveal any further structural detail, and in particular, the expected subfibrillation is not visible. Other structural features can, however, be made out with phase contrast by stretching the fresh fibril; a pale segment, transected by the *Z*-membrane, now appears, being sharply differentiated from the darker zone that is transected by the *M*-membrane (figure 67, plate 20). In polarized light with polarizing filter, the dark zone appears anisotropic, *Z* and *M* isotropic (figure, 68, plate 20).

The greatest internal differentiation of the fresh fibril is seen when we examine it in polarized light with filter and combined with phase contrast. In many of the fibrils, but not in all, the expected subfibrils appear, but with variable degrees of clarity. Figure 69, plate 20, is a photograph taken under these conditions. The fibrils are very faint, but are none the less recognizable on close scrutiny. The pale zone to either side of *Z*, above described for the stretched fibril, is here apparent, and is therefore not a consequence of stretching. Midway between successive *Z*-membranes an unexpectedly thick *M*-membrane is visible.

We turn now to the fixed preparation. Here the use of good fibrillar fixatives, such as alcoholic Bouin, or A.A.T., is necessary, the thorax being completely bisected to admit the fixative rapidly; very thin sections (1μ) are desirable though not essential; finally, Heidenhain's iron haematoxylin alone can be relied upon to stain with the required degree of sharpness. If these precautions are observed, then at least in the bee and other Hymenoptera the subfibrils can be consistently revealed.

The following description relates partly to the honey-bee (*Apis mellifica* L.) and partly to a local species of wild bee (*Halictus speculiferus* Cockerell), which responds even better to histological method. Examined in very thin cross-section (1μ), the coarse fibrils at once display their component fine fibrils (figures 65, 75, plates 20 and 21). Often these tend to aggregate, and cannot then be separately resolved; but in the sharpest preparations, the discrete character of the fibrils is unmistakable. The photographs have been taken in monochromatic green light to obtain good resolution. In the clearest pictures eight to ten fibrils can be counted. They are embedded in an amorphous ground substance, optically different from the surrounding sarcoplasm, and binding the fibrils into units. An investing membrane cannot be seen.

Longitudinal sections are shown in figures 70, 71, 76, 77, 78, plates 20 and 21. Figures 70 and 71 are from the honey-bee, the others from *Halictus speculiferus*. All were fixed *in situ*; in

figure 76 the fibrils are considerably stretched. In all cases the composite character of the coarse fibrils is evident; there is, however, indication of distortion by the fixative, for the fibrils, particularly in figure 70, are less regularly disposed than in the fresh fibril shown in figure 69. The *Z*-membrane is always very clear, the thinner *M*-membrane also being visible in figures 70, 76, 77 and 78.

Further analysis by the aid of fixed preparations is necessarily suspect, having in mind the artifacts that are likely to ensue. In places where the *Z*-membrane has stained only feebly, we get the impression that the delicate fibrils actually pass through the membrane, implying for the membrane itself a fenestrated structure. This is seen in the right bottom corner of figure 76, and there is an indication of it in the smaller fragment shown in figure 71.

A feature that appears commonly in fixed preparations is a pale zone to either side of the *Z*-membrane. Close inspection of figure 81, plate 21, shows that this pale zone is due to differential staining of the delicate fibrils, and not to the intervening ground substance. A similar pale zone has been referred to above for the fresh fibril after stretching. These data seem to be complementary, and suggest an altered structure of the delicate fibril in the neighbourhood of the *Z*-membrane, as shown in ordinary muscle by polarized light (Introduction, §F).

The absence of recognizably shortened fibrils is a most noteworthy feature of this form of muscle; certainly neither electrical stimulation nor immersion in irritant fixatives can exert an effect that is general for other forms of muscle. Long ago Merkel (1872) noticed that when isolated fibrils were immersed in egg-white, spontaneous shortening gradually ensued. I have had no difficulty in confirming this. The shortening, which begins after about an hour, is gradual and irreversible, and not a normal process; and it is, as Merkel saw, attended by typical 'striation reversal' (figure 79, plate 21). When a piece of wing muscle, immersed in albumen, is examined at the onset of shortening (after about an hour immersion) the fibrils begin to show alined dots in the position usually referred to as *N*. They are visible in figure 78, plate 21. Their infrequent occurrence in normal (?) muscle is referred to above (Introduction, §F). An *N*-band is actually visible in the fresh fibril shown in figure 69, plate 20, and, as figure 68 suggests, lies at the line of junction of the isotropic and anisotropic bands.

There is yet another appearance that we commonly find in the fixed tissue, but this has no parallel in the fresh fibre; in place of the dark *M*-band we see a clear unstained line, so that the coarse fibril may even appear as a string of unconnected segments (figures 71, 80, plates 20 and 21). The possibility comes to mind that these appearances may represent isometrically contracted fibrils, with limited 'striation reversal'. But fibrils that are undeniably contracted do not show these clear bands (figure 79). Probably, therefore, the appearances, which are really very abundant, are a gross technical fault, the entire *M*-membrane having failed to stain. In confirmation of this is the frequent occurrence of similar appearances in forms of wing-muscle where true shortening occurs (figure 86, plate 21). But we are then left with the dilemma that in coarse fibrillar wing-muscle we cannot recognize a state of shortening. In some preparations fixed *in situ*, I have found in the bee that the inter-*Z* distance over a long succession of striations is only $2{\cdot}3\mu$, instead of the usual $2{\cdot}7\mu$. Whether this represents a state of slight shortening is uncertain.

Finally, we may refer to preparations made by inadequate technique. Figure 72, plate 20, is an example, the tissue having been fixed in non-alcoholic Bouin's fluid. Here is the familiar picture of the coarse fibrils, devoid of any visible subfibrillation. The fibrils are slightly stretched, and severely shrunken; *M* is unstained but *Z* is just visible where it protrudes a little beyond the margin of the fibrils.

(ii) *Sericesthis pruinosa* (Coleoptera, fam. Scarabaeidae)

When stimulated with an induction coil this muscle, like that of the bee, does not seem to contract. The frequency of wing beat is unknown; in other Coleoptera it is fairly high (Sotavalta 1947).

The fibrils are coarse and closely resemble those of the bee. The range of thickness is from 2·5 to 3μ; the inter-*Z* distance is 2·3μ. The tissue responds well to histological method, the component fibrils being readily displayed (figure 82, plate 21). There is only one notable difference between these coarse fibrils and those of the bee: when isolated they usually immediately shorten to about two-thirds their normal length.

(iii) *Eurymela distincta* (Homoptera, fam. Jassidae)

The fresh fibrils range from 3·3μ to as much as 5μ in thickness, with an average thickness of 4μ. The inter-*Z* distance is about 3μ. Figure 83, plate 21, shows the fresh isolated fibril, seen with phase contrast. With appropriate fixation and staining the usual structural detail, already described for the bee, is shown, though not so consistently.

The isolated fibrils, unlike those of the bee, show a marked tendency to shorten immediately. Figure 84, plate 21, shows a group of such shortened fibrils, examined fresh in polarized light with polarizing filter and phase contrast; the component fine fibrils are visible with surprising clarity; observe also the 'striation' reversal, and compare in this respect with the fixed and stained fibrils of the bee, shown in figure 79, plate 21.

In the bisected *Eurymela* the living muscle, unlike that of the foregoing type, shortens considerably with direct faradic stimulation, even though it does not pass into strong tetanus. It is necessary, in making the observation, to guard against a spurious contraction, due to shortening of the underlying leg muscles. The insect is sufficiently large to permit removal of the dorsal longitudinal muscles, exposing the various tergo-sternals. When minute electrodes are directly applied to these muscles, their individual contraction is readily seen. But although the muscle is thus able to shorten considerably, the familiar contractile waves of other types of muscle are never found in fixed preparations.

(iv) *Siphanta acuta* (Homoptera, fam. Flatidae); *Cyclochila australasiae* (Homoptera, fam. Cicadidae)

In both these types there is much deformation of the thoracic wall during flight. In *Siphanta* the frequency of wing beat attains 37 to 41/s, in *Cyclochila* 25/s (stroboscope determinations). The response of the wing musculature to faradic stimulation is quite different from that of the foregoing types, the muscle immediately passing into violent tetanus that severely distorts the thorax if bisected.

In *Siphanta acuta* the fresh uncontracted fibrils measure a little under 1μ in thickness. Simple manipulation does not disrupt the tissue into its elementary fibrils, as is the case with the types so far described; if, however, the muscle is placed for several days in

a refrigerator, the sarcoplasm loosens, and isolated fibrils are then easily obtained. Figure 85, plate 21, shows two such fibrils, one of which is considerably stretched; they have been photographed with phase contrast, which accounts for the clarity of the *Z*-membrane, and, in the stretched fibril, of the *M*-membrane.

Analysis by fixation is attended by the usual uncertainty of technique. The commonest picture is one in which the fibrils appear as narrow rods, without evidence of subfibrillation, with *Z* usually well defined, and *M* completely unstained, so that it may even give the appearance of a clear line transecting the whole fibre (figure 86, plate 21). At other times *M* stains quite deeply (figure 87, plate 21). The varying appearances recall those already described for coarse fibrillar muscle.

Even with good fibrillar fixatives there is much shrinkage of the fibrils, and neither in longitudinal nor in cross-section do they show even a sign of subfibrillar structure. But with a minimum of shrinkage, the presence of two to four component fibrils can be made out (figure 88, plate 21). It is the presence of three subfibrils that commonly, with imperfect fixation, gives a triangular form to the transected fibril.

In the cicada *Cyclochila australasiae* the fibrils are very similar to those of *Siphanta acuta*. The appearance of the fixed resting fibril is shown in figure 138, plate 24. Here again the composite character of the fibril is evident if fixation is good; figure 89, plate 21, shows a cross-section, figure 90, plate 21, at lower magnification, a longitudinal section.

Both species exemplify a form of muscle in which considerable specialization in the direction of wing-muscle has taken place. Yet neither falls into the category of coarse fibrillar muscle. The fibres of *Cyclochila* are described in detail below (p. 296); those of *Siphanta* are similar to those of *Scolypopa*, selected as a type later (p. 317). They contract strongly with electric stimulation, and when tissue fixed *in situ* is examined, we are not surprised to find typical contraction zones, with inter-*Z* distance reduced below two-thirds resting length, and with the usual striation reversal. Figure 91, plate 21, shows an example from *Cyclochila*.

(v) *Caedicia olivacea* (Orthoptera, fam. Tettigoniidae)

With faradic stimulation the muscle goes into strong tetanus. Flight is weak, with a wing frequency of about 8 to 10/s (simple inspection during flight).

The wing-muscle fibres in this insect exemplify the last transition to normal unmodified trunk muscle, with fibrils collected into muscle columns, recognizable in cross-section as Cohnheim areas (figure 94, plate 22). The presence of sparse intracellular tracheae, and of a richer supply of sarcosomes, are the only distinctive features of the wing-muscle fibres, and even the latter feature is not wholly distinctive, for in the prothorax many fibres are quite rich in sarcosomes.

The fresh fibre does not dissociate into fibrils, but dissociation is readily achieved by placing the tissue for a few days in a refrigerator. Figure 93, plate 22, shows a group of four such fibrils, seen with phase contrast. They measure less than 1 μ thick, and display conspicuous *Z*-bands. *M* cannot be seen, but is readily visible in fixed tissue. The inter-*Z* distance is 4 μ.

This tissue responds unusually well to fixation, which brings out within the fibrils the presence of three to five subfibrils, bound into a unit by amorphous ground substance (figure 95, plate 22).

Discussion

The foregoing observations can leave little doubt as to the status of the coarse Siebold fibrils of higher insects; plainly they are neither muscle columns nor giant myofibrils, but sarcostyles as above defined, and can be directly derived, through the lower grades of insects, from the sarcostyles of normal trunk muscle. But it is evident that their evolution has been attended by important changes in physiological property, and we have to inquire to what extent these directly determine the very exceptional function of wing-muscle in the higher orders of insects.

(i) *Structure.* Here we may usefully discuss a few additional points. We have seen (Introduction, p. 231) that in unspecialized muscle the myofibrils of a sarcostyle are separately connected by tonofibrillae to the chitin. Does this mechanical arrangement hold also for the coarse Siebold fibril? Morison (1928) has already shown that the thick sarcostyle is connected by numerous tonofibrillae to the chitin; whether the tonofibrillae are individually connected with its component myofibrils is more difficult to determine. This arises from the fact that the end-to-end fusion takes place by a deeply staining thickening, and that such adjacent thickenings, at least in fixed preparations, commonly merge into a single mass, to which on one side the tonofibrillae, and on the other the myofibrills, are attached. When this takes place the end-to-end fusion is obscured, but is at once seen if the thickenings remain apart.

Consider figure 102, plate 22 (from a bee *Paracolletes* sp.); this represents a fragment of a section along the insertion of a wing-muscle fibre on to the chitin, the attachment of five of the coarse sarcostyles being in focus. The point of fusion of tonofibrillae with sarcostyle is marked, in each case, by a deeply staining mass, which is the above-described clumped row of thickenings; but the clumping is not complete, and therefore the direct connexion of individual tonofibrillae and myofibrils is seen wherever the focus is properly adjusted. Figures 100, 101, plate 22, show two examples from a wasp *Scolia bimaculata*; here fusion of adjacent granules is less complete, and the end-to-end attachment is therefore plainly evident; note, incidentally, how the fanning out of the tonofibrillae spreads the myofibrillae at the ends of the sarcostyles. Usually this is confined to the terminal compartment of the sarcostyle, i.e. beyond the last *Z*-disk. Occasionally, however, it causes a splitting of the sarcostyle; in figure 192, plate 27, for example, from a jassid *Erythroneura ix*, the split has extended over thirteen cross-striations.

The early work of Merkel (1872) on the coarse sarcostyle is important, for it gave the first indication of the *Z*- and *M*-disks, and showed the 'reversal of striation' at spontaneous shortening. The experiments of Merkel on the swelling of sarcostyles led him to infer the presence of an ensheathing membrane, and several authors have actually claimed to see it (Schäfer 1891; Jordan 1920*b*; Marcus 1921). In sections through the fresh sarcostyle we do, indeed, get this appearance (figure 64, plate 20); it is almost certainly a diffraction effect, arising from closure of the iris diaphragm that is necessary to reveal the colourless sarcostyle. In the fixed and stained sarcostyle I cannot see this membrane (figures 65, 75). The statement of Marcus (1921) that the sarcostyle is tubular, and that the *Z*-disks are rings (hoops) in the sheath is inconsistent with all the evidence given above; the statement of von Ebner (1918) that the *Z*-disks are rings of sarcosomes, is also not supported.

In the well-known work of Schäfer (1891, 1912) the sarcostyle is envisaged as a giant highly differentiated fibril; the middle part of each 'sacromere' is 'formed of a mass of chromatic substance which is everywhere perforated by longitudinal tubules' that open into a fluid reservoir at the ends of the 'sarcomeres'. At contraction the fluid is said to be drawn into the middle porous part, producing thickening and therefore shortening of the sarcomere. The well-known illustration of three wasp sarcostyles (stretched, relaxed and shortened) still reproduced with approval in many histological treatises, is, as Jordan (1920*b*) has shown, essentially incorrect; movement is actually in the reverse direction (striation reversal); there are no visible tubules; and finally the hope that the giant sarcostyle might reveal the secret of contraction will not be fulfilled, for it is not a single myofibril at all.

(ii) *Function.* We may surmise that the character of the fibrillation is in some way bound up with the form of wing movement, and this conjecture is supported when we find that occasionally an abdominal muscle, functioning as accessory wing muscle, actually assumes the character of coarsely fibrillated (Siebold) muscle (see below, Delphacidae, p. 323). But where the correlation lies has yet to be determined.

Correlation with speed or endurance of flight may at once be excluded: psyllids, thrips and aphids with coarse sarcostyles, are amongst the feeblest flyers; on the other hand, among thinly fibrillated forms, usually with weak flight, are the butterflies, of which some are renowned for their wandering habits.

The coarse type of sarcostyle is found in the higher orders of insects where wing beat is predominantly rapid, even though individual members may be exceptional in this respect (Diptera, Hymenoptera, Coleoptera, Thysanoptera, Psocoptera and many Hemiptera); but in the Lepidoptera, many Homoptera, and in all the lower orders (Orthoptera, Dermaptera, Ephemerida, Embioptera, Perlaria, Isoptera, Mecoptera and Neuroptera), the sarcostyles are thin, and here wing movement is predominantly slow. Yet the exceptions are sufficiently important to exclude a complete dependence of frequency of wing beat on the character of the fibril. For instance, a small jassid that I have examined (*Erythroneura ix*) has a wing frequency of 29 to 31/s, but its sarcostyles are mostly thick, and tipulids with thick sarcostyles also have a low frequency (Voss (1913–14), gives 44/s for one species; for others Sotavalta (1947) gives frequencies of a similar order). Opposed to this we find some hawk-moths with relatively high frequency of wing beat (Marey (1874) gives 72/s for one species), yet the sarcostyles are thin.

The real correlation seems to lie with the character of the contraction, coarse fibrillation tending to appear when conditions are becoming isometric. Certainly in fixed preparations of coarsely fibrillated muscle we never see the zones of contraction, with *Z*-disks drawn together, that are general for thinly fibrillated muscle. There is, however, no implication that thickness of sarcostyle itself conditions isometric contraction; for instance, in the jassid *Erythroneura ix* (described below), the metathoracic muscles, unlike the mesothoracic, have rather thin sarcostyles (figure 92, plate 21, and compare, for example, with figure 86); but zones of contraction, of the thin sarcostyle type, this muscle does not display.

The extremes of adaptation to the two types of contraction, with and without pronounced shortening, are given by the Lepidoptera on the one hand and by the Diptera and Hymenoptera on the other.

(*a*) *Lepidoptera.* Here the frequency of beat ranges from 2 to 3/s in large tropical *Ornithoptera* to over 70/s recorded for a hawk-moth. In butterflies particularly, there is violent deformation of the thorax during flight. In the hawk-moth this is much reduced; examined under a binocular microscope with wings rapidly vibrating, it shows a pronounced trembling rather than the violent deformation of the butterfly thorax.

If the wings are clipped, the frequency usually rises; for example, in the hawk-moth *Hippotion scrofa* a steady beat of 57/s rose to 66/s when two-thirds of each wing were clipped.*

The following are some observations on the effect of direct electric stimulation of the bisected thorax. A 'square-wave' stimulator, permitting accurate grading of frequency, was used. The recording was purely visual. In the butterfly *Heteronympha merope*, with 6 to 7 beats/s, the wing muscle in the bisected thorax responds by separate twitches up to 8 to 10 stimuli/s. With progressive increase in frequency of stimulation, the muscle passes progressively into tetanus, the attendant wing beat diminishing in amplitude as it increases in frequency, up to 36/s, when summation is complete. In *Hippotion scrofa* (57/s) complete summation is delayed to 70/s, though even at 30/s fusion is advanced. In the intact thorax, with thoracic wall exerting full tension on the muscle, the upper limit might be higher. The rough data seem to show: (*a*) that to meet the needs of higher frequency contraction, the property of the muscle itself changes, without visible change in fibril structure; (*b*) that apparently only the peaks of contraction of the two sets of antagonistic

* This result came as a surprise, having in mind Roeder's (1951) finding that in the moth *Agrotis*, clipping diminished frequency of beat. The point has become important because of the supposed difference in reaction to 'unloading' displayed by insects with very fast (Diptera) and with relatively slow wing beat. Measurements on other species have, almost without exception, given results similar to those on the hawk-moth, as follows:

	normal	clipped (half of wing removed)
Noctuidae		
Sideridis ewingi	47	53 to 55
S. unipuncta	52	65
	52	59
	51	58
Proteuxoa aspersa	49	55
Caradrina passalota	21	23 to 26
C. paratorna	21	30
Peripyra sanguinipuncta	20	26
	20	27
Neuroptera		
Chrysopa signata	23	27
	24	29
Notochrysa insignis	21	33 ($\frac{4}{5}$ clipped)
Homoptera		
Siphanta acuta	41	46
	41	47
Isoptera		
Callotermes sp.	24	26
	21·6	23·2 to 20
	18	17

The readings were made with a stroboscope, the insect being held by the abdomen with forceps. Some individuals had to be discarded owing to erratic wing beat. Where steady readings were obtained, the readings before and after clipping are surprisingly constant. A single specimen of *Elhamma australasiae* (Hepialidae) gave a diminished beat (58 to 53) after clipping. *Callotermes* gave an initial rise, which in one individual fell off to a diminished beat due apparently to fatigue; in the third individual the initial rise was not found.

muscles, working in rapid antiphase, are used in the hawk-moth to produce the up- and downstroke of the wing, implying therefore a considerable degree of isometric contraction. Heidermans (1931) has already shown that in dragonflies the wing muscles are in almost complete tetanus when stimulated at the frequency with which the wings beat in flight, implying for these muscles also a high degree of isometric contraction.

(*b*) *Diptera.* With clipped wings the frequency of beat rises (Buddenbrock 1919; Roch 1922; Chadwick & Williams 1949); but in view of the foregoing results for 'slow' muscle, this can no longer be regarded as distinctive for high-frequency muscle. A more striking difference is the unresponsiveness of the latter to direct electric stimulation. This was noted by Kölliker (1850) and later by Kühne (1862), the latter, on that ground, even disputing its muscular character. Weismann (1862) attributed it to injury to which this form of muscle is prone; yet there should be little difficulty in at least exposing the muscle without damage. Merkel (1872) then found the spontaneous contraction of the sarcostyle, since when Kühne's finding has been a riddle.

This apparent unresponsiveness of the muscle seems to be bound up with the high-frequency isometric character of the contraction. I have examined this point further on *Eristalis tenax* (wing beat 139 to 175/s, Sotavalta 1947). If the thorax is bisected, electrodes applied directly to the longitudinal muscles do not give a visible contraction, even when the frequency of stimulation is raised to 1000/s or more, i.e. far above the frequency of wing beat. If an appropriate fragment of the scutum is removed with a razor, the ends of some of the dorso-ventral fibres are exposed; when such a fly, with wings rapidly vibrating, is watched under a binocular, we are surprised to find the detached muscle fibres participating in the contraction, but without retraction. It seems that the almost isometric contraction to which the wing muscles are subject in the intact thorax is not imposed by the rigidity of the thoracic wall, but is a property of the muscle itself. If, in another fly, one wing together with the surrounding chitin is removed with a razor, the action of the dorso-ventral muscles can be watched under a binocular. The intact wing in such a fly will perform normal flying movements, and in this the injured side of the thorax participates; yet movement of the muscle is scarcely perceptible. In *Eristalis* I have, on one occasion, had a full view of the longitudinal muscles contracting in the bisected insect, and with the single wing in full vibration. Usually the bisected insect is completely paralyzed; in this instance normal and prolonged flying movements could be repeatedly started by electric stimulation of the brain. Examined under a binocular, and despite full exposure, movement in the muscle was barely perceptible. With direct electric stimulation the movement ceased instantly.

Contraction of this minute order, and differing wholly from summated contraction, will, if obtained by direct stimulation, be very difficult to see, especially if tautness on the muscle is lessened by bisection. The most we can expect to see is minute movement of the chitin, similar to that observed in flight. In examining this point the dorso-ventral muscles are best avoided, for they include, in most Diptera, a single muscle (tergo-trochanteral) that is not of wing-muscle type (see below, Diptera, p. 333). The longitudinals are more suitable: if in a decapitated insect a small piece of chitin is removed from the tip of the thorax, these muscles can be directly stimulated through the hole by minute electrodes. Close inspection of the hinder end of the thorax, and especially of the scutellum,

then shows very minute movements in the chitin. There is also some wing movement, but this is necessarily damped by some contraction of the dorso-ventral muscles.

A local species of large tipulid fly (*Plusiomyia olliffi*) lends itself better to these experiments. When flying in a glass jar, the wing beat is only 42/s. If examined under a binocular with the wings rapidly vibrating, deformation of the thoracic wall is, for a member of the Diptera, surprisingly large. In the bisected thorax direct electric stimulation of the longitudinal muscles here causes easily visible contraction, as was indeed expected.

We conclude, then, that as isometric conditions developed in the thoracic mechanism, the wing musculature accommodated itself by gradually restricting its ability to shorten, rather than by balancing the tension of antagonistic muscles, as in the hawk-moth. This seems to be something new, and indeed unique, in striated muscle, and it is the coarse type of sarcostyle that displays it. We do not infer that thin sarcostyles would be incompatible with high-frequency contraction, nor that coarseness of sarcostyle determines it; but coarse fibrillation must be incompatible with anything but the most restricted shortening if this is to be rapid, the mobility of a viscous cylinder being necessarily promoted by its longitudinal subdivision. But plainly the molecular structure of the myofibrils is also a factor in determining high-speed contraction. Other factors may also be involved; thus Pringle (1949) has made the strange observation that in the blow-fly frequency of the motor volley falls far short of the frequency of motor response, the myogenic rhythm being apparently imposed by the resonating frequency of the thoracic wall.

Reference is necessary to yet another point. Isometric contraction with fairly slow wing beat need not imply an absence of rapidly propagated shortening, even of some magnitude, for a zone of shortening may stretch a relaxed portion of the fibre; we have seen above that in *Siphanta acuta* even with a wing frequency of about 40/s local shortening is considerable. For high-frequency vibration we should expect this factor to be reduced to a minimum, possibly by starting the contraction at various intervals along the muscle (see below, Diptera, p. 340), and perhaps also by a change in the physical property of the sarcostyle; and in this respect it is noteworthy that muscle with coarse sarcostyle, even despite slow wing beat (e.g. *Erythroneura ix*, 30/s), never shows the contraction zones that are inevitably found in the less specialized fibre.

B. *The sarcoplasm*

This is still best examined in sections of the fresh frozen tissue.

The transition to the sarcoplasm of specialized wing-muscle may conveniently be described by reference to the muscles of *Caedicia olivacea* (Orthoptera), already examined in regard to sarcostyle structure. The Cohnheim pattern in fibres of the prothoracic muscles has already been described and illustrated (figure 44, plate 19); we see in the cross-section rather elongate polygonal Cohnheim areas, merging into a lamellar pattern around the margin of the fibre, and with only few sarcosomes in the interstitial substance. The same pattern is evident in the fibres of its flying muscles, though obscured to a varying degree by the rich sarcosome content (figure 94, plate 22). In comparing these photographs it is necessary to emphasize the very considerable difference in picture of sarcoplasm shown by fibres even of the same muscle. The granulation is often much coarser, in the flying muscles, than figure 94 indicates; and among the prothoracic muscles exceptional fibres are even

almost as rich in sarcosome content as any fibre in the flying muscles. Indeed, the presence of sparse intracellular tracheae is the only constant feature distinguishing the flying from non-flying muscles (a fragment of trachea is shown in figure 94 marked by an asterisk). The sarcosomes, in this species, are not orientated with respect to the cross-striation, though a tendency to longitudinal orientation is usually evident. But in other, even primitive Orthoptera (e.g. mantids), a cross-alinement appears, and is especially evident in Acridiidae, where, however, a Cohnheim pattern is not recognizable (cf. figure 125, plate 23). In all the Orthoptera the sarcoplasm seems to have a markedly viscous character; no Brownian movement is visible, and neither teasing nor pressure can isolate the sarcostyles.

In higher insects with coarse sarcostyles, a change in consistency of sarcoplasm is at once evident. If a mass of wing muscle from a fly is placed under a cover-glass, even gentle pressure on the glass suffices to squeeze the sarcosomes from the framework of sarcostyles, and it is then readily seen that the sarcoplasm, with sarcosomes floating freely in it, has a fluid character. A fluid sarcoplasm would promote the mobility that is needed for high-speed contraction, even when this is virtually isometric. The tendency of wing muscle to dissociate into sarcostyles was one of the first known of its exceptional properties, and it is doubtless the fluid sarcoplasm that is the cause. A Cohnhein pattern is, of course, absent, and the sarcosomes are transversely alined.

A chemical study of the sarcoplasm is beyond the scope of this work. Wigglesworth (1949) has shown that the glycogen is confined to it. Enzymatic studies on the sarcosomes, by Watanabe & Williams (1951), have already been referred to above.

C. *The cross-membranes*

The reason for inferring the presence of cross-membranes in wing muscle is the same as for other forms of striated muscle, for the sarcostyles are organized within the fibre to produce the pattern of cross-striation.

Despite the unreliability of available methods, the demonstration of the *Z*-membrane, at least in the lower orders of insects, does not present any real difficulty. Examples will be given below for various species; it is sufficient here to say that not in a single species has the *Z*-membrane failed to appear in at least some preparations. A special problem arises in cases of unusual sarcostyle pattern, where there are wider spaces between the groups of sarcostyles; does the membrane traverse the spaces? Examination of this point is best deferred till the special cases arise (see below, *Cyclochila*, p. 296; *Scolypopa*, p. 317). The reader who is still suspicious that the *Z*-membrane may be a product of fixation should examine figure 98, plate 22. This represents a frozen section of the fresh wing-muscle fibre of a butterfly, *Heteronympha merope*, seen in polarized light and filter, and with phase contrast, the method bringing out unusually well the *Z*-membrane completely traversing the interfibrillar spaces.

Turning now to muscle with coarse sarcostyles, there is little difference of opinion concerning a *Z*-component for at least the sarcostyle itself. Merkel (1872) was the first to describe it, and it can be seen readily in almost every instance even in the fresh tissue (figures 63, 66, 83, 278, 279, but not 276, 277). But close inspection of the fresh intact fibre, even with the *Z*-disks of the sarcostyles sharply defined, seldom gives even a hint of

an interfibrillar connexion; thus in figure 63, plate 20, there is only very doubtful evidence of such a membrane. Thulin (1915), who has specially examined this point, is even prepared to distinguish the coarse fibrillar muscle from other types of fibre, as 'grundmembranlos', and general experience would seem to confirm this. The difficulty of the cross-alinement of striations, however, remains. My own experience affirms the presence of the membrane, though its demonstration with available methods is capricious; indeed, it is best seen when fibril fixation is only indifferent. Consider figure 73, and compare with figure 63; it is plain from the photograph that selective fixation and staining, while suppressing the sarcosomes, can, in the bee, bring out the interfibrillar *Z*-membranes, even with some clarity; and careful scrutiny of the photograph will show that the appearance is not an illusion arising from sarcostyle thickening at the level of *Z*. Careful focusing reveals it, moreover, as a fenestrated rather than completely unbroken membrane, the fenestration producing the occasional gaps that appear in the photograph (the abundant intracellular tracheae, suppressed in the fixed preparation, also imply some degree of fenestration of the membrane). Is the *Z*-membrane marginally connected with the sarcolemma, as in other types of fibre? Figure 74, plate 21, answers this in the affirmative.

The position with regard to the *M*-membrane remains unsatisfactory. In the coarse (Siebold) sarcostyle, *Mf* is usually distinct (see above), and is indeed more consistently seen in the fresh fibril than after fixation (figures 66 to 69, 83, plates 20 and 21). But general experience would be against the presence of an interfibrillar (*Ms*) connexion; indeed, there does not seem to be any claim in the literature of the existence of such a membrane. It has therefore come as a surprise to find, especially in material in which the sarcostyle fixation is only indifferent, that a complete *M*-membrane, even transecting the interfibrillar spaces, is really present. Figure 96, plate 22, shows the membrane in a wasp (*Scolia bimaculata*), and as figure 97 shows, the membrane is marginally connected with the sarcolemma; in either case it is a very thin membrane, alternating with the thicker *Z*-membranes. I have seen it also in the honey-bee.

In lower insects, with thin sarcostyles, the *Mf*-disk, visible in fresh tissue (e.g. figure 85, plate 21), is commonly seen also after fixation (figures 87, 90), though it is as likely to remain unstained; in the latter case it is then a sharp clear line transecting the *Q*-band, and if the fibrils are not displaced, it even gives the illusion of a clear line completely transecting the fibre (figure 86). In this particular species (*Siphanta acuta*), I have occasionally had evidence of an *Ms*-membrane transecting the spaces between the sarcostyles; it is visible in figure 87, though fainter than *Zs*. In cicada muscle also, the whole *M*-band usually remains a clear unstained line (figure 183, plate 26); yet in some material fixed in Carnoy's fluid, and stained by a method (Willis's silver technique, see above) that would seem quite unsuited to the purpose, an *M*-membrane of most exquisite delicacy, midway between successive *Z*-membranes and completely transecting the interfibrillar spaces, has appeared (figures 147, 148, plate 25).

It is hard to escape the conviction that a specific technique, once discovered, will consistently bring out the *Ms*-membrane; how much of the dense intracellular tracheal network do non-specific methods reveal? Yet a difficulty will arise in certain species where the sarcosomes seem to be alined at the level of the *M*-band.

D. *Sarcolemma*

There is general agreement that at least in unspecialized wing muscle of lower insects and of Lepidoptera, a sarcolemma is present (van Gehuchten 1886; Kölliker 1888; Kielich 1918; Jordan 1919). I have had no difficulty in confirming this, the membrane being most readily demonstrated in sections cut from the unfixed frozen thorax. The usual attachment to the cross-membranes of the fibres is easily seen in good fixed preparations.

The discordant opinion regarding the existence of this membrane in coarsely fibrillated muscle has already been set out above (Introduction, §A): it involves the question as to whether an investing membrane is always present, and whether, if present, it is a derivative of the muscle fibre, or a web spun out between the investing tracheae (Holmgren 1908). In all species that I have examined, a sarcolemma is present; this includes the bee and *Drosophila* where their presence has been denied. It is best seen in cross-sections of frozen unfixed tissue; figure 74, plate 21, shows it from a fixed preparation of bee muscle. Commonly after fixation the sarcolemma shrinks against the sarcostyles, and then cannot be seen in longitudinal sections. In transverse sections there is really no difficulty in seeing it, even though it is sometimes rather faint; figure 263, plate 31, shows it, for instance, from a cross-cut fibre of *Rutilia*.

Is it a true sarcolemma, or a web between the tracheae? There is really no evidence to support the latter contention. In good preparations, for example, it shows the familiar 'festooning' where it is attached to the *Z*-membranes (figures 74, 97, plates 21 and 22), and this seems to be incompatible with any extraneous origin from tracheae. That tracheae do often form webs investing the muscle fibres is certainly true; thus in the large fibres of many Diptera the network, after hardening in alcohol, can easily be stripped away from the underlying muscle fibre. But in proper histological preparations in which tracheae and sarcolemma have been stained simultaneously, the distinction between the two is at once evident. Figure 99, plate 22, for example, shows a section that grazes along the surface of a wing-muscle fibre from a bombiliid fly. It has been prepared by the Da Fano method, and as is often the case with this technique, the sarcolemma is made visible by a fine deposit of silver granules. The tracheae stand out sharply, but there is certainly no indication that the sarcolemma is a web between them, as envisaged by Holmgren. For further discussion, see below, Diptera.

The remainder of this paper is devoted to the structure and development of representatives of several insect orders, with a view to discovering a possible evolutionary trend from the archaic to the specialized type of wing-muscle.

The problem will be much clarified if we try to envisage the background on which the wing musculature may be supposed to have evolved. It is generally agreed by morphologists that wings arose as tergal expansions of the thoracic segments, which served initially as simple gliding organs, but became movable wings by acquiring an articulation with the thoracic wall. Deformation of the thorax was the means whereby the wings were moved, and for this the existing trunk musculature must necessarily, in the first place, have been called into service. The required changes in muscle attachments or in the structure of skeletal parts that enable muscles to assume such altered function are familiar to morphologists (see Weber 1933; Snodgrass 1935); our problem now is to discover the attendant

changes in the muscular tissue itself, as it prepares to accept the additional role of wing vibrator.

The character of the old thoracic musculature before the acquisition of wings can now only be guessed at from its structure in surviving Apterygota. A member of the Thysanura will be chosen as type, entailing an examination of its myology, and of manner of growth of the muscles in the enlarging nymph.

The Orthoptera, as the most primitive surviving winged insects, will then be examined in some detail. Here the thoracic musculature has undergone a minimum of structural adaptation to flight; and since the new function is not added till after the last moult, we should expect the appropriate changes to be deferred till the end of the nymphal period. From a comparative study of the different families of Orthoptera, information will then also be sought regarding the incipient evolution of a specific wing musculature, and the histological changes that attend it. Throughout the Orthoptera growth of the muscles in the nymph has proved to be due to fibre proliferation, with a minimum of fibre enlargement, and therefore differs radically from the myogenesis of higher insects.

It is plain that a thoracic muscle can accept only to a limited degree the additional role of a wing vibrator, and for that reason flight among Orthoptera, even at its best, is crude. A muscle that begins to subserve the function of high-frequency contraction, culminating in higher orders in restricted ability to shorten, must become increasingly unsuited, and finally useless, in its initial role. How has the musculature responded to these growing demands?

Evidence will be given below that such a crisis has arisen at least once in the evolution of the wing musculature; such, at least, is the interpretation that we may reasonably place on the evidence disclosed by Homoptera, where a diversity of myogeneses has come to light. Fibre proliferation in the nymph, inherited from the lower insects, is here retained to a variable degree; but to this a new process has become added—the progressive incorporation of free myoblasts into the cleavage products. Initially these myoblasts are reserve cells within the functional nymphal fibres. In other forms they are, from the beginning, independent of such, and in the young nymph start gradually, fibril by fibril, to build up the fibres of which the muscles are composed. It is but a short step then to the most specialized development of all, that of higher metabolic insects, where the whole process, with multitudes of myoblasts co-operating, is deferred to the onset of metamorphosis.

Much exacting work will be needed before any connected account of the genesis of the wing musculature in general can be given. The present paper gives a fairly detailed statement for Orthoptera, a less complete study on Homoptera, in which only certain families have so far been examined, and a short account of Diptera. I hope to treat other orders in later papers.

THYSANURA

Ctenolepisma longicaudata Esch.

This is the common 'silver fish' of domestic dwellings. Relevant external features are: (i) the large tergal scutes, one in each thoracic segment; (ii) a conspicuous 'subcoxa' with two supracoxal arches (anapleurite and coxopleurite of Snodgrass), which morphologists envisage as the forerunners of the pleurites of the pterothorax.

(1) *Myology* (figure 1)

A. *Tergal muscles*

(i) *Median dorsal longitudinal muscle* (*m.d.l.*), a wide sheet of muscle, just under the tergal wall; posterior attachment in each segment to antecosta; anterior to median tergal wall, the laterally placed fibres veering outwards to attach to antecosta; dorsal flexor of thorax.

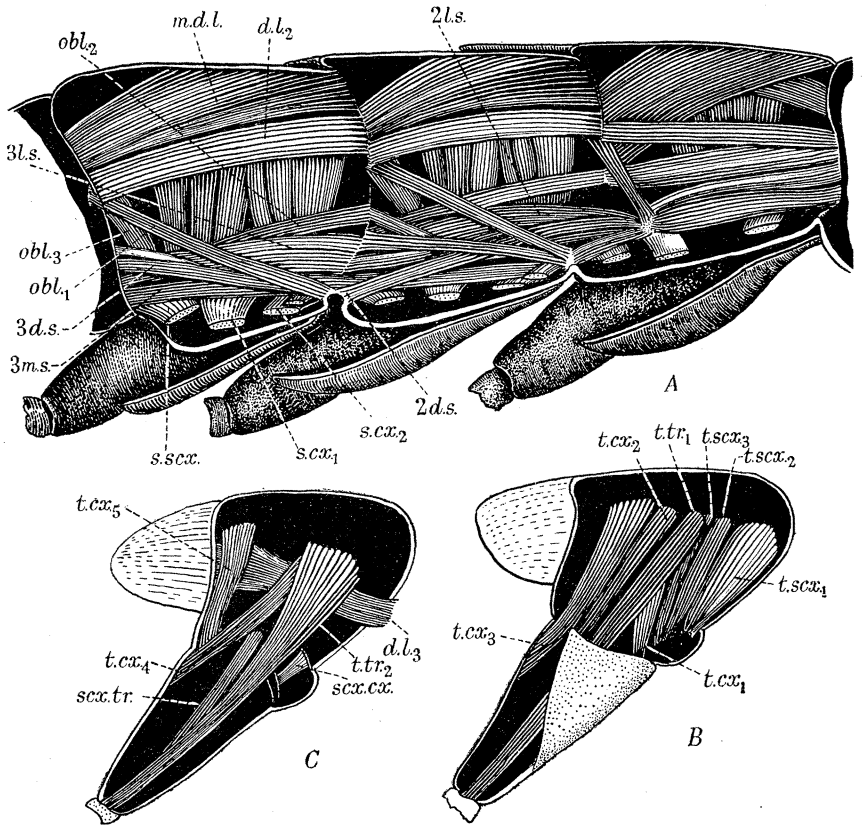


FIGURE 1. *Ctenolepisma longicaudata.* Thoracic musculature. *A*, median bisection of thorax; *B*, metathorax, longitudinal muscles removed; *C*, the same, after removal of muscles shown in *B*.

(ii) *Second dorsal longitudinal muscle* (*d.l.*$_2$), a strong muscle passing from antecosta to antecosta (and in prothorax to head), and lying ventro-lateral to (i); dorsi-flexor of thorax.

(iii) *Third dorsal longitudinal muscle* (*d.l.*$_3$), a strong muscle, lateral to the dorso-ventrals, and attached behind to the scutes.

B. *Sternal intersegmental muscles*

These are of considerable complexity, but will be described only briefly, as they are not relevant to the problem of wing muscle. The principal muscles are:

(iv) A lateral metathoracic muscle (3 *l.s*), passing from spina to spina, and median to it (3 *m.s*), a single long muscle, traversing, in addition, the full length of the mesothorax, to the spina of which it is attached. To its side is a mesothoracic muscle (2 *l.s*), attached in front to the prothoracic furca, from which, in turn, two muscles pass to the head (unlabelled in figure).

(v) Diagonally running muscles (3 *d.s*, 2 *d.s*) attached, as shown, either to the spina or to the prothoracic furca.

C. *Oblique trunk muscles*

(vi) In the mesothorax and metathorax a muscle ($obl._1$) that passes upward from the spina to the succeeding tergite.

(vii) In each segment a muscle ($obl._2$) passing up from the spina to the preceding tergite (or, in prothorax, to head).

(viii) In each segment a muscle ($obl._3$) passing obliquely up from the furca to the succeeding tergite.

D. *Dorso-ventral muscles*

These are brought to full view by removing the tergal and sternal intersegmental muscles. We may conveniently distinguish those that are directly attached to the leg (leg muscles) and those that operate on the leg through the subcoxa (subcoxal muscles).

(*a*) *Leg muscles*

(ix) *First tergo-coxal muscle* ($t.cx._1$), arising laterally from the tergite, and attached to the inner anterior rim of coxa; adductor of coxa.

(x) *Second tergo-coxal muscle* ($t.cx_2$), attached to hind inner rim of coxa; adductor of coxa.

(xi) *Third tergo-coxal muscle* ($t.cx._3$), arising just lateral to (x); lower attachment within coxa; coxal remotor.

(xii) *Fourth tergo-coxal muscle* ($t.cx._4$), arising from the tergite laterally to (x) and (xi), and attached to rim of coxa; probably a coxal remotor.

(xiii) *Fifth tergo-coxal muscle* ($t.cx._5$), arising laterally from tergite, and attached to outer rim of coxa; abductor of coxa.

(xiv) *First tergo-trochanteral muscle* ($t.tr._1$), arising just in front of (x), and passing down the coxa to its attachment on the trochanter; extensor (?) of trochanter.

(xv) *Second tergo-trochanteral muscle* ($t.tr._2$), lateral to the former; abductor of leg, and flexor (?) of trochanter.

(*b*) *Subcoxal muscles*

(xvi) *First tergo-subcoxal muscle* ($t.scx._1$), anterior origin on tergite, lower attachment to anapleurite of subcoxa.

(xvii) *Second tergo-subcoxal muscle* ($t.scx._2$), arising behind (xvi), and attached to coxopleurite.

(xviii) *Third tergo-subcoxal muscle* ($t.scx._3$), arising behind (xvii), and attached to the subcoxal membrane.

E. *Subcoxal leg muscles*

These are laterally placed, and comprise:

(xix) A minute muscle (*scx.cx*), passing from coxopleurite to outer rim of coxa.

(xx) A muscle (*scx.tr.*) arising from coxopleurite, and attached to trochanter.

F. *Sternal leg muscles*

(xxi) A long and powerful muscle (*s.scx.*) arising from the spina, and passing forward to its attachment within the subcoxa; it opposes the action of the tergal-subcoxal muscles.

(xxii), (xxiii) Two muscles ($s.cx._1$, $s.cx._2$) that arise from the sternal wall and from the spina respectively, and are attached within the coxa, on which they have a remotor action.

Certain of the above muscles, such as the median and second dorso-laterals, and the dorso-ventral muscles associated with the base of the leg, find their counterpart in the orthopteran pterothorax. Whether the subcoxal muscles can be the forerunners of the pleural muscles of Orthoptera seems very doubtful. A subcoxal muscle (xx) operating on the trochanter is, however, suggestive of the third basalar of lower Orthoptera, which has a similar attachment (see below).

(2) *Histology of muscle fibres and their development during the nymphal period*

The muscle fibres are of the radial lamellar type, with an axial core of nucleated protoplasm. In the lamellae (sarcostyles) the expected subfibrillation could not be demonstrated.

The *Z*-membrane, transecting the interlamellae, is frequently visible. I have failed to see any interlamellar connexion.

The sarcosomes lie mainly within the axial sarcoplasm; short rows of granules are also found in variable amount among the lamellae.

Enlargement of the muscle during growth of the nymph takes place solely by fibre enlargement, without any attendant increase in fibre number; for example, the median dorsal longitudinal muscle comprises, both in the young nymph and in the adult, some twenty muscle fibres. During fibre enlargement the lamellae increase in width and in number. 'Branched' lamellae are very abundant throughout the period of fibre enlargement, but not in the minute nymph, and this is consistent with the view that the new lamellae arise by division of pre-existing lamellae.

ORTHOPTERA

This group is primarily of importance for the light it can still throw on the initial adaptation of the thoracic musculature to flight.

Several very detailed studies have already been made of orthopteran myology, by Voss (1905) and Carpentier (1923) on gryllids, and by Snodgrass (1929) on the acridiid *Dissosteira*. The present work extends information to the more primitive families. It does not aim at a complete description of the whole musculature, but is confined to those muscles that can be considered either as actual or as potential wing muscles. This should suffice to disclose any evolutionary trend within the group that could underlie the improvement in flight found in higher Orthoptera. The further question will then arise of structural adaptation of the musculature during the nymphal period, and of the attendant histological changes.

BLATTIDAE

Blattella germanica L.

Though very agile this insect has only feeble powers of flight.

(1) *Myology* (figure 2)

The following description covers the tergal, dorso-ventral, pleurotergal, pleural and wing-adjustor muscles, though whether they are all used in flight is uncertain; other muscles, such as the sternals, though illustrated, are not described, since they can play no direct role in flight.

Mesothoracic muscles

A. *Tergal muscles*

(i) *Median dorsal longitudinal muscle* (*m.d.l*), attached to the small phragmas, but surprisingly poorly developed; a median component ends midway along scutum. Weak wing depressor.

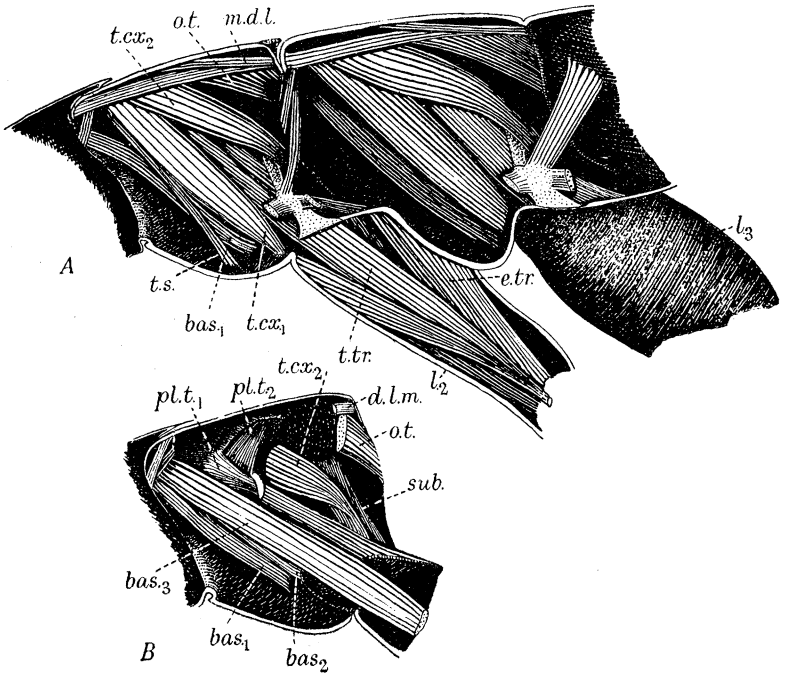


FIGURE 2. *Blattella germanica*. *A*, musculature of pterothorax, seen in median bisection; *B*, mesothorax, median muscles removed; l_2, l_3, second and third legs.

(ii) *Oblique tergal muscle* (*o.t.*), which from its attachment along the hinder lateral phragma of the segment spreads medially upward, and is inserted over a considerable area of the scutum lateral to (i). The direction of the fibres is such that its contraction must arch the scutum, and it is therefore the main wing depressor. In other families of Orthoptera this muscle is much diminished in size.

B. *Dorso-ventral muscles*

(iii) *Tergo-sternal muscle* (*t.s*), an unexpectedly thin muscle, attached below to basisternum; presumably a weak indirect wing levator.

(iv) *First tergo-coxal muscle* (*t.cx.*$_1$), large, attached below to a small sclerite in membrane at base of coxa. Apart from its promotor action on coxa it could, with fixed coxa, be used as an indirect wing levator.

(v) *Tergo-trochanteral muscle* (*t.tr*), an exceptionally large muscle, passing down the long coxa, where it is reinforced by numerous fibres arising from the upper rim of the coxa itself; it is attached to the trochanter, and besides acting as depressor on the latter, could, with fixed trochanter, act as indirect wing levator. Its antagonist, with respect to its action on the trochanter, is a powerful muscle on the opposite side of the coxa, and arising from the coxal rim (indicated by *e.tr* in figure 2*A*).

(vi) *Second tergo-coxal muscle* (*t.cx.*$_2$), a large muscle passing obliquely across the segment, and attached to hinder rim of coxa; apart from a remotor action of the coxa, it could serve as indirect wing levator.

C. *Pleural muscles**

These are seen when the dorso-ventrals are removed.

(vii) *First basalar muscle* (*bas*$_1$), attached below to membrane above rim of coxa, and above to the large basalar sclerite; weak 'direct' wing depressor.

(viii) *Second basalar muscle* (*bas*$_2$), a thin sheet of muscle lying behind (vii); lower attachment around rim of coxa, upper to basalar sclerite; weak 'direct' wing depressor.

(ix) *Third basalar muscle* (*bas*$_3$), an unusually large muscle attached above to basalar sclerite, and below by a tendon in common with (v) to the trochanter. Apart from its depressor action on trochanter it could be a 'direct' wing depressor.

(x) *Subalar muscle* (*sub*), a single short muscle, attached above to subalar sclerite and to the surrounding membrane, and below extending a short distance into the coxa, to the outer wall of which it is attached. Apart from its adductor action on coxa, it is a 'direct' depressor of the wing.

D. *Wing adjustor muscles*

(xi) *First pleuro-tergal muscle* (*pl.t.*$_1$), arising from pleural apodeme, and attached to scutum above wing base; tilts wing up.

(xii) *Second pleuro-tergal muscle* (*pl.t.*$_2$), similar origin, and inserted above on thin chitin around allula; presumably flexes wing.

In the metathorax the muscles are similar but considerably larger; a notable difference is the absence of the tergo-sternal muscle.

(2) *Histology*

Most of the muscles, whether or not they are flight muscles, are composed of fibres of the radial lamellar type (figures 103, 105, plate 22). In some exceptional muscles with thin fibres, these have a weakly developed lamellar pattern around the margin of the fibre, enclosing a dense inner core of evenly distributed cylindrical sarcostyles. In the coxal promotor (first tergo coxal) muscle, described in detail below, this type of fibre forms a subsidiary bundle with the principal bundle composed of radial lamellar fibres (figure 105).

The sarcolemma is best seen in frozen cross-sections of the fresh fibre. The muscle nuclei are distributed at random, sometimes just below the sarcolemma, at other times among the sarcostyles (figure 103). Even in the lamellar type of fibre there are considerable numbers of cylindrical sarcostyles in the axial core of sarcoplasm.

* There is considerable disagreement in the literature on the function of the pleural (epipleural) muscles that are attached to the epipleural sclerites. In a large grasshopper, *Caedicia valida*, I have been able to stimulate the muscles by microelectrodes inserted through minute incisions in the overlying chitin. When the coxa is rigidly fixed, stimulation separately of basalar and subalar muscles has, in either case, the effect of depressing the extended wing, and of tilting the costal margin down. They are therefore depressor-rotator muscles. Extension and expansion of the wing seem to be the function of many muscles. Stimulation of the dorsal longitudinals, in addition to depressing the wing, certainly has this effect; so has stimulation of the dorso-ventrals, particularly those attached to the prescutum.

Of the cross-membranes the *Z*-membrane, marginally attached to the sarcolemma, and completely traversing the interlamellar spaces, can actually be seen in the fresh fibre; this is shown, for example, in figure 104, the focus being adjusted to fall just below the sarcolemma, an air-filled trachea on the surface of the fibre being simultaneously focused. I have not been able to see a complete *M*-membrane, though the *Mf*-band of the sarcostyles is usually fairly distinct in fixed tissue. The inter-*Z* distance is about 2·7 μ, reduced to about half at full contraction.

Sarcosomes, best seen in the fresh fibre, are present in the axial sarcoplasm, and often invade the marginal zone of lamellae, where they lie in short longitudinal rows.

The tracheal supply to the muscles is not rich, and the tracheae do not penetrate the sarcolemma. I cannot give any data regarding the innervation.

The foregoing description applies also to the prothoracic muscles; indeed in *Blattella*, where flight is very feeble, the muscle fibres of the pterothorax do not seem to have developed any special structural features to meet their new function.

(3) *Development*

Apart from the minute wing adjustor muscles there is, in *Blattella*, only one muscle that is an exclusive wing muscle. This is the tergo-sternal (indirect levator) of the mesothorax. The others are either (i) trunk muscles functioning in the nymph, and adapted to flight by change in their attachments (median dorsal longitudinals, oblique tergals, pleurals), or (ii) muscles that combine an action on the leg with their presumed role in flight, but without undergoing change in attachment (dorso-ventral muscles acting on leg base).

In *Blattella* the skeletal changes that impart a new action to functional nymphal muscles are: (i) formation of the slightly developed phragmas, which convert the tergal muscles exclusively into indirect wing depressors; (ii) partial separation of a basalar sclerite from the episternum, and the formation of a flexible membrane at the upper end of the epimeron, enabling the epipleural muscles to pull on the wing base. These changes take place in the last nymphal instar.

We have here to consider the development: (i) of those muscles that are actually functioning during the nymphal period (the first tergo-coxal of the mesothorax will serve as example); (ii) of the pure flight muscle, non-functional in the nymph (tergo-sternal of mesothorax). A cross-section through the muscles is shown, at low magnification, in figure 105. The tergal-coxal is fairly large, and consists of about 160 fibres, of which about 60 are of the above-described narrow type, and form a distinct bundle along its median surface (this is easily seen in figure 105). The tergo-sternal is very thin, and comprises only twelve fibres, and is seen in figure 105 lying alongside the tergo-coxal.

(i) *Tergo-coxal muscle.* This consists, in the minute first-instar nymph, of about sixty fibres, the narrow fibres numbering about thirty-five, and comprising a disproportionately large part of the muscle (figure 106). In the larger fibres the pattern of marginal lamellate sarcostyles is already evident; these enclose an axial core of nucleated sarcoplasm, to which, for the present, the nuclei are confined. As the nymph enlarges the muscle grows apace, the principal bundle soon outstripping the bundle of thin fibres. This initial growth of the muscle is due almost entirely to enlargement of its fibres. Within the fibres the lamellae increase in numbers and in width, and the nuclei begin to move from the axial

sarcoplasm into the outer zone of lamellae, giving to the fibre a markedly adult appearance (figure 107, plate 22).

In nymphs that are still less than half-grown, a process of fibre cleavage now begins, a surprisingly large proportion of fibres being affected at the same time. Figure 107 shows a cross-section through part of the muscle, at this stage of development. At a hasty glance one gets the impression of fracturing of fibres through defective sectioning; actually the rather disordered appearance is due entirely to fibre cleavage. Several fibres have split into three, others into two, and others again are evidently approaching cleavage. This cleavage necessarily upsets the sarcostyle pattern of the fibres, especially when cleavage into three takes place; but the disordered pattern is quite transitory, and is speedily repaired. A fuller description of these processes is given below for *Periplaneta*, where the cleavage is more active than in *Blattella*.

(ii) *Tergo-sternal muscle.* In early nymphs the rudiment of this muscle is a mere delicate wisp, lying alongside the tergo-coxal muscle, and visible only in microtome sections; not till late in the nymphal period can it be recognized in dissections. Initially it measures not more than about 5μ in cross-section (figure 106). It is enclosed in a delicate membrane (sarcolemma), and contains a small number of delicate fibrils which are just perceptibly cross striated. Nuclei are scarce. As the nymph grows, the muscle rudiment, plainly a rudimentary muscle fibre, enlarges (figure 107), its fibrils increasing in number, and displaying more distinct cross-striation. Within the sarcolemma the fibrils now become grouped into separate columns, which in the later nymph are recognizable as daughter fibres, with a marginal zone of lamellae, and enclosing an axial core of sarcoplasm (figure 108). The entire muscle, comprising some 12 fibres, is therefore derived from a single rudimentary fibre.

Other Blattidae

I have made a brief examination of the musculature of several other blattids, viz. *Periplaneta americana*, *Panesthia australis*, *Oniscosoma granicollis* and *Platyzosteria analis*, the last-named a wingless form.

The muscles are very similar to those of *Blattella germanica*; in *Periplaneta* and *Panesthia* they are relatively larger. *Platyzosteria analis* shows some instructive differences related to its flightless condition: the median dorsal longitudinal muscles are even weaker than in *Blattella*, for there are no phragmas; the oblique tergals, which are the principal wing depressors in *Blattella*, are present, and even strongly developed, but here must function as flexors of the thorax; finally, the mesothoracic tergo-sternal is, as expected, absent.

Regarding the histology of the fibres, in *Periplaneta* they resemble those of *Blattella*, having a radial lamellar pattern (figure 112, plate 23), with rows of sarcosomes and without intracellular tracheae. In *Panesthia* and *Oniscosoma* the sarcostyles are cylindrical, and are evenly dispersed through the fibre. In *Panesthia* there is a considerable development of intracellular tracheae.

In *Periplaneta americana*, of which I have had a fairly complete series of nymphs, fibre cleavage proceeds as in *Blattella*, but more actively. The first tergo-coxal muscle consists, in this species, of about 500 fibres with radial lamellar pattern, grouped into about forty bundles, and there is an accessory bundle of thin fibres, as in *Blattella*. The lamellate fibres

take their origin from about forty nymphal fibres, each producing, by repeated cleavage, one of the fibre bundles. The early development is as in *Blattella*, the fibres enlarging and their nuclei moving into the marginal zone of lamellae. Fibre cleavage becomes active in the 10–15 mm nymph, all the fibres being affected. A typical picture of the fixed tissue is shown in figure 109, plate 22. To the left, cleavage of a single fibre into four is seen, while below it are two smaller fibres whose sarcostyle pattern indicates derivation from the parent fibre above. To the right of this fibre group is another group of five, presumably a progeny of a single parent fibre, but more completely separated. Stages in repair of the sarcostyle pattern are seen in all the daughter fibres, a layer of small lamellae making its appearance along the inner surface of the original lamellae, from which they then separate to enclose an axial cavity occupied by sarcoplasm. These small lamellae may arise either by separation from the inner ends of the large lamellae, or by migration from a proliferating zone along the lateral margins of the cleft fibre.

The fixed preparation directs attention almost exclusively to the sarcostyles, and gives scarcely a hint as to how the cleavage is brought about. On the latter point frozen sections of the fresh fibre are instructive, for they show also the sarcoplasm and particularly sarcolemma. Figure 110, plate 22, represents such a section, from a 15 mm nymph, and is strictly comparable with figure 109. It is at once evident that fixation causes severe shrinkage, and that the large spaces between the daughter fibres are unreal. Fixation also has the effect of almost suppressing the visibility of the sarcolemma; the fresh tissue now shows that each daughter fibre is invested by this sheath. In places we find that the sarcolemma has extended only partly into a cleaving fibre—an example is shown in the fibre marked with an asterisk—and the impression is then given that in some way cleavage is due by actual ingrowth of the sarcolemma. Usually the cleavage is in the plane of the lamellae, i.e. it is radial, and this could suggest that cleavage was initiated by separation of lamellae. But in exceptional cases the cleavage completely transects the lamellae, i.e. it is tangential, and this is more consistent with an active cleavage by sarcolemmal ingrowth. An example of this is shown in the fibre marked with two asterisks, representing a group of six daughter fibres that have arisen by cleavage from a parent fibre. The fibre concerned is shown at higher magnification in figure 111, plate 22; close scrutiny of thep hotograph shows that the cleavage (two asterisks) has transected about twenty adjacent lamellae.

Sections of the fresh tissue also show the air-filled tracheae. The tracheal network becomes more intricate as the muscle enlarges, and as figure 110 shows, soon penetrates among the daughter fibres.

MANTIDAE

Orthodera ministralis Fabr.

This is the common green mantis of eastern Australia, measuring when adult up to 5 cm in length. Only occasionally does it take to the wing, flight being weak and of brief duration.

(1) *Myology* (figure 3)

The disposition of the muscles is remarkably similar to that of blattids. In figure 3 the muscles of the metathorax are shown with a minimum of dissection, so that some of the laterally placed muscles are not visible; in the mesothorax these have been displayed by

removing some of the medial muscles. The lettering is as in figure 2. We may here dispense with a detailed account of the muscles, and refer only to some points of general interest.

In common with blattids we find very poorly developed median dorsal longitudinal muscles, but a strong oblique muscle in the mesothorax (2 *o.t*), though feebly developed (3 *o.t*) in the metathorax. The first tergo-coxal is unusual in arising in two separate parts from the tergal wall. The rather weak tergo-sternal (pure flying) muscle of the mesothorax is similar to that of blattids, as are also the three basalar muscles, of which one operates

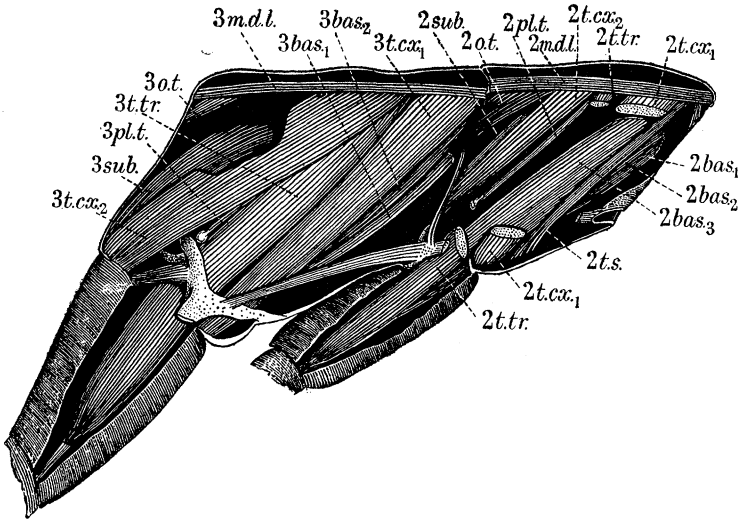


FIGURE 3. *Orthodera ministralis*. In the mesothorax the laterally placed muscles have been exposed by removal of medial muscles.

on the trochanter by a tendon in common with the tergo-trochanteral muscle. The mesothorax in figure 3 is dissected to show both these muscles (2 bas_3, 2 *t.tr*). There is a single subalar muscle in each segment, with lower attachment to the membrane at the base of the coxa, and not, as in blattids, within the coxa. A pleuro-tergal muscle (*pl.t*) with lower attachment to the pleural apodeme occurs in both segments, but is only poorly developed, especially in the metathorax. In blattids it is absent; in Tettigoniidae (see below) it is found in the mesothorax only, but is there powerfully developed, being one of the principal wing levators.

The strong development of the metathoracic muscles is in marked contrast to their rather weak development in the mesothorax. But even in the metathorax the tergal muscles are weak, so that in the mantis the principal flying muscles are the epipleurals and dorso-ventrals.

In another species that I have dissected (*Tenodera australasiae*) the musculature is very similar; dorsal longitudinal wing depressors are here completely absent however.

(2) *Histology*

The fibres are polygonal in cross-section, measuring 0·02 to 0·05 mm in width. The sarcolemma is easily seen in the fresh frozen section as a smooth delimiting membrane. The nuclei lie mostly just under the sarcolemma.

The sarcostyle pattern, as shown in fixed preparations, has a marginal zone of thin lamellae, enclosing a large central zone of thin cylindrical sarcostyles, many of which are

grouped as in figure 115, plate 23. In the cylindrical sarcostyles two to four myofibrils can at times be distinguished.

Frozen sections give a just perceptible hint of a Cohnhein interstitial network. Sarcosomes are very abundant and of moderate size, and are disposed in two irregular transverse rows, one to either side of the *Z*-membrane. Jordan (1916) has already described transverse alinement in a species of mantis.

The tissue has as usual a very rich tracheal supply. Blindly ending tracheae penetrate in considerable numbers through the sarcolemma but undergo comparatively little branching, so that the intracellular tracheal supply is never rich.

(3) *Development*

The picture is here remarkably similar to that of Blattidae, even though in the adult there are pronounced differences in fibre structure. In the newly hatched nymph the fibres are cylindrical, measuring about 5 to 8μ across, the sarcostyles forming a narrow marginal zone of lamellae, enclosing an axial core of nucleated sarcoplasm, as in *Blattella*.

Initially there is the usual fibre enlargement, the lamellae growing in width and in number till the nymph reaches about 2 cm in length. Fibre cleavage then ensues, all the fibres soon becoming involved. Figure 116, plate 23, represents a fragment of a cross-section of the metathoracic tergo-trochanteral muscle. The appearances already described for blattids are here again evident, but do not require further description.

The rate of cleavage soon outstrips that of muscle enlargement and so the fibres become much reduced in diameter, and in the advanced nymph we find a preponderance of quite diminutive fibres. A grouping of fibres in accordance with their derivation from a single parental fibre remains in evidence.

In the last nymphal instar the fibres undergo much enlargement, and tracheae now penetrate through the sarcolemma.

PHASMIDAE

Ctenomorpha marginipennis Gray

The adult measures about 15 cm in length, with wings not more than $2\frac{1}{2}$ cm. It is unable to fly and is barely able even to move the wings.

(1) *Myology*

The disposition of the muscles is greatly affected by the excessive elongation of the body. This specialized musculature can have little relevance to the problem at hand, and will therefore be described only briefly, and without illustration.

In the mesothorax the muscles are confined to the hinder end of the segment where the legs and wings are located. There is a short and very weak median longitudinal, and a considerably stronger oblique, both ending a little in front of the wings. A weak tergosternal is present, and might perhaps be used to raise the tegmen. The first tergo-coxal is surprisingly large, and spreads forwards some distance along the segment. The tergotrochanteral is weak, but the second tergo-coxal (coxal remotor) is remarkably large, spreading forward so that in the bisected thorax most of the other muscles are obscure. The basalar sclerite is a ridge of chitin fused with the pleural ridge, so that the basalar

muscles cannot possibly pull on the wing; these comprise the usual set of three, the third operating on the trochanter. The subalar sclerite is attached by flexible membrane to the wing base, suggesting that the single subalar muscle might possibly pull on the wing (wing flexor?). The presence of a short stout pleuro-tergal muscle arising from the pleural ridge is noteworthy. There is also a short axillary wing extensor.

In the metathorax there is a weak median longitudinal and a longer oblique. The dorso-ventrals have an almost horizontal disposition across the segment. They comprise a single thin coxal promotor (first tergo-coxal), lying alongside a much larger tergo-trochanteral, behind which is the second tergo-coxal. There are only two basalars, both attached to the coxal rim, and without action on the wing. The subalar is long and might perhaps pull on the wing. Pleuro-tergal and axillary muscles are present, as in the mesothorax.

(2) *Histology and development*

For reference muscle I select the coxal remotor of the metathorax. It comprises in the adult some 200 fibres, with a surprising diameter range of 0·02 to 0·14 mm. The nuclei of the fibre lie mostly just under the sarcolemma. The marginal sarcostyles are lamellar, and enclose an axial core of cylindrical sarcostyles. Apart from this the fibre presents nothing noteworthy.

In the 3 cm nymph, which is the earliest stage that I have examined, thirty to forty fibres are present, of average diameter 0·02 mm. The fibrillar pattern is as in the adult; the nuclei lie in the depth of the fibre. Though I have only a few nymphal specimens, mostly rather young, these suffice to show that the fibres multiply by longitudinal cleavage as in other Orthoptera.

GRYLLOTALPIDAE

Gryllotalpa australis Er.

This is the common Australian mole-cricket. The adult measures up to 3 cm in length, and has only the feeblest power of flight, which is restricted to a few yards. The tegmina are short, and when rubbed together emit in the male the melodious call that is familiar in late summer. The female also is said to produce a faint call. Whether the tegmina are used in flight is uncertain.

(1) *Myology* (figure 4)

This is in some respects highly specialized; the powerful sternal musculature derived from two long median apodemes at once obtrudes in the bisected thorax. Muscles arising from it operate on the legs; but they are not relevant to the present work, and do not need description. In the metathorax they have been removed to display the flying muscles more fully.

In the mesothorax the longitudinal tergal muscle (2*l.t.*) (depressor of tegmen) is short; it is not an intersegmental muscle, having its anterior attachment to an apodeme from the scutum. The expected tergo-sternal muscle is absent, the only dorso-ventrals being the first and second tergo-coxals (promotor and remotor of coxa) and the tergo-trochanteral (depressor of trochanter), all strongly developed. The three basalars present nothing unusual; as in most other Orthoptera, the third operates on the trochanter. There is a single subalar, with lower attachment to the membrane above the coxal rim. Two

axillaries are present, arising from the pleural ridge, one attached to the first axillary sclerite, the other to the third.

In the metathorax the wing muscles are larger. The median dorsal longitudinal, attached to the phragmas, is a thin sheet of muscle. There is a single weak oblique. The first and second tergo-coxals present nothing unusual; the tergo-trochanteral is exceptionally thin. There are three basalars, attached above to the basalar sclerite and of these the third is thin and is inserted on the trochanter. There is a single subalar, and two axillaries attached as in the mesothorax.

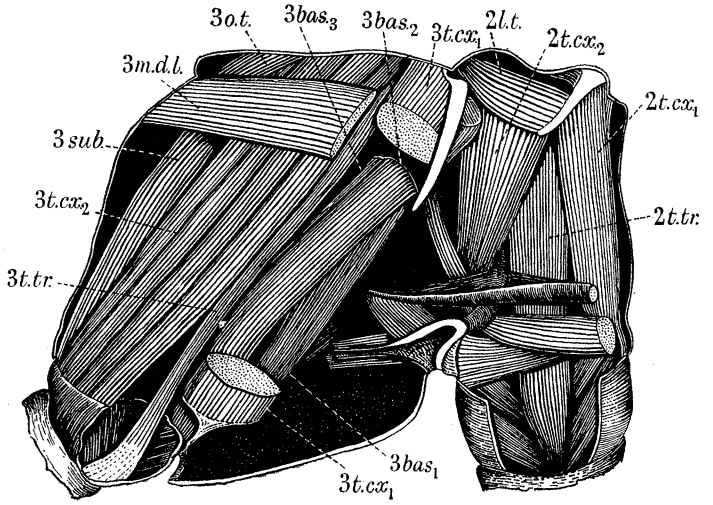


FIGURE 4. *Gryllotalpa australis.*

(2) *Histology*

A most unusual feature in the metathoracic but not mesothoracic muscles is the presence of spacious confluent tracheal vessels within the muscles (figure 113, plate 23); large internal tracheae are described below in the muscles of other Orthoptera, but I have nowhere met such a multitude of large vessels as in *Gryllotalpa*.

In fibre structure there are marked differences between the two groups of muscles. In both there is a very narrow marginal zone of lamellae, and a large axial column of cylindrical sarcostyles. But in the metathorax the fibres are surprisingly thin, averaging about 0·015 mm across, while in the mesothorax they are nearly three times as wide. The mesothoracic fibres show a coarse Cohnheim pattern. The nuclei lie mostly just under the sarcolemma; in the metathoracic muscles they are surprisingly abundant. Both groups of muscles are rich in sarcosomes, which are disposed in an irregular double row, one to either side of the cross-membrane, but easily dislodged by manipulation. Only in the mesothoracic muscles do tracheae enter the fibres; they are abundant, but most end blindly.

(3) *Development*

I have obtained a considerable series of nymphs, ranging from 9 mm up to the final instar; first instars are lacking. For convenience I shall describe the tergo-trochanteral muscle of the mesothorax. This consists in the adult of about 250 fibres, with the above-described fibre structure. In the 9 mm nymph only thirty-two fibres are present, some

evidently the product of recent cleavage. In the 15 mm nymph there are about seventy-six fibres, and at 20 mm the full number is present, though with greatly reduced diameter. Initially there is much fibre enlargement, but as the above counts show, this is soon followed by fibre cleavage. Both the sarcostyle pattern and the process of fibre cleavage are very similar to those of *Acridopeza*, described in detail below. We may therefore dispense with a detailed description; it will suffice to give a single illustration of the cleavage picture (figure 114, plate 23).

In the last nymphal instar the fibres undergo much enlargement, acquire a richer sarcosome content, and become permeated with tracheae.

GRYLLIDAE

Gryllus servillei Sauss.

This is the common Australian 'black cricket', the adult measuring about 1 in. in length. The metathorax is large and contains most of the flying muscles; the reduced tegmina produce the familiar cricket 'chirp', but whether they are used in flight is uncertain.

(1) *Myology*

Since a very detailed account for another species of *Gryllus* has already been given by Voss (1905), a short statement, without illustration, will suffice. In the mesothorax the musculature is weak. The dorsal longitudinal is short and feeble. The tergo-sternal of other Orthoptera also is weak, and is attached below, not to the sternum, but to a ridge along the lower margin of the episternum (from Voss's account this is not so in *G. domesticus*). The first basalar, usually connected to the coxal rim, is similarly attached. The third basalar, as in other Orthoptera, is a depressor of the trochanter. There is only a single subalar.

In the metathorax the longitudinal muscle ranges from a weak to, in some individuals, a fairly strong muscle having its attachment to the phragmas. The first and second tergo-coxals (coxal promotor and remotor respectively) are exceptionally large (Voss describes two coxal promotors in *G. domesticus*, but there is only one in *G. servillei*). A second coxal remotor is present, and is, in some individuals, even a powerful muscle. The tergo-trochanteral is surprisingly thin. The three basalars present nothing unusual. There is a single large subalar, attached below to the membrane at the base of the coxa.

(2) *Histology*

The fibres are polygonal in cross-section, and unusually thin, measuring 18 to 40μ in width. The nuclei lie just below the sarcolemma. The sarcostyle pattern closely resembles that of the mantis *Orthodera*. Sarcosomes are abundant and are transversely alined. A *Z*-membrane, but not *M*, is visible. The inter-*Z* distance is about 3μ at full relaxation.

Tracheae penetrate the sarcolemma, but do not branch extensively, so that the tracheal supply is not rich; most of them end blindly.

(3) *Development*

I select for reference muscle the second tergo-coxal of the metathorax, a large muscle comprising between 1200 and 1300 fibres. In the minute nymph it consists of about thirty

fibres, averaging about 7 to 8μ in diameter, with radial lamellae that enclose an axial nucleated core of sarcoplasm, and closely resembling the fibres of young blattids already described. The usual fibre enlargement now ensues. When the nymph reaches about 7 mm in length fibre cleavage begins, and the picture closely resembles that of *Blattella*. In the 10 mm nymph cleavage is proceeding actively; the general picture still rather closely resembles that of blattids, and we may therefore dispense with illustration. In the advanced nymph the pattern of the cross-section changes a little, the marginal lamellae becoming less distinct, while an abundance of cylindrical sarcostyles forms axially by splitting off from the lamellae.

TETTIGONIIDAE

Acridopeza reticulata Guérin

This is the 'mountain grasshopper' of southern Australia. The male is fully winged; the female, without wings, has short tegmina, and makes no endeavour to use them in flight. Both sexes are sluggish creatures, the female being heavily built, about 3 cm in length, the male more slender and shorter, but with long wings extending back 2 cm beyond the abdomen. Even the male takes only rarely to the wing, and seems to have only weak powers of flight. I have selected this species for very detailed description because the musculature of the wingless female provides most instructive comparison with that of the winged male.

(1) *Myology* (figure 5)

Only those muscles that are potential wing muscles are considered, viz. the tergal, dorso-ventral, pleuro-tergal, pleural and wing adjustor muscles. There is the usual doubt as to whether individual muscles that operate on the leg are also used in flight; in this species, however, marked enlargement of the muscle in the male may be taken as almost certain evidence that it functions in double capacity. A few other muscles that are conspicuous in dissection are shown in figure 5, but are not labelled.

In the mesothorax the musculature is more complex than in the metathorax, but the muscles are smaller. They comprise:

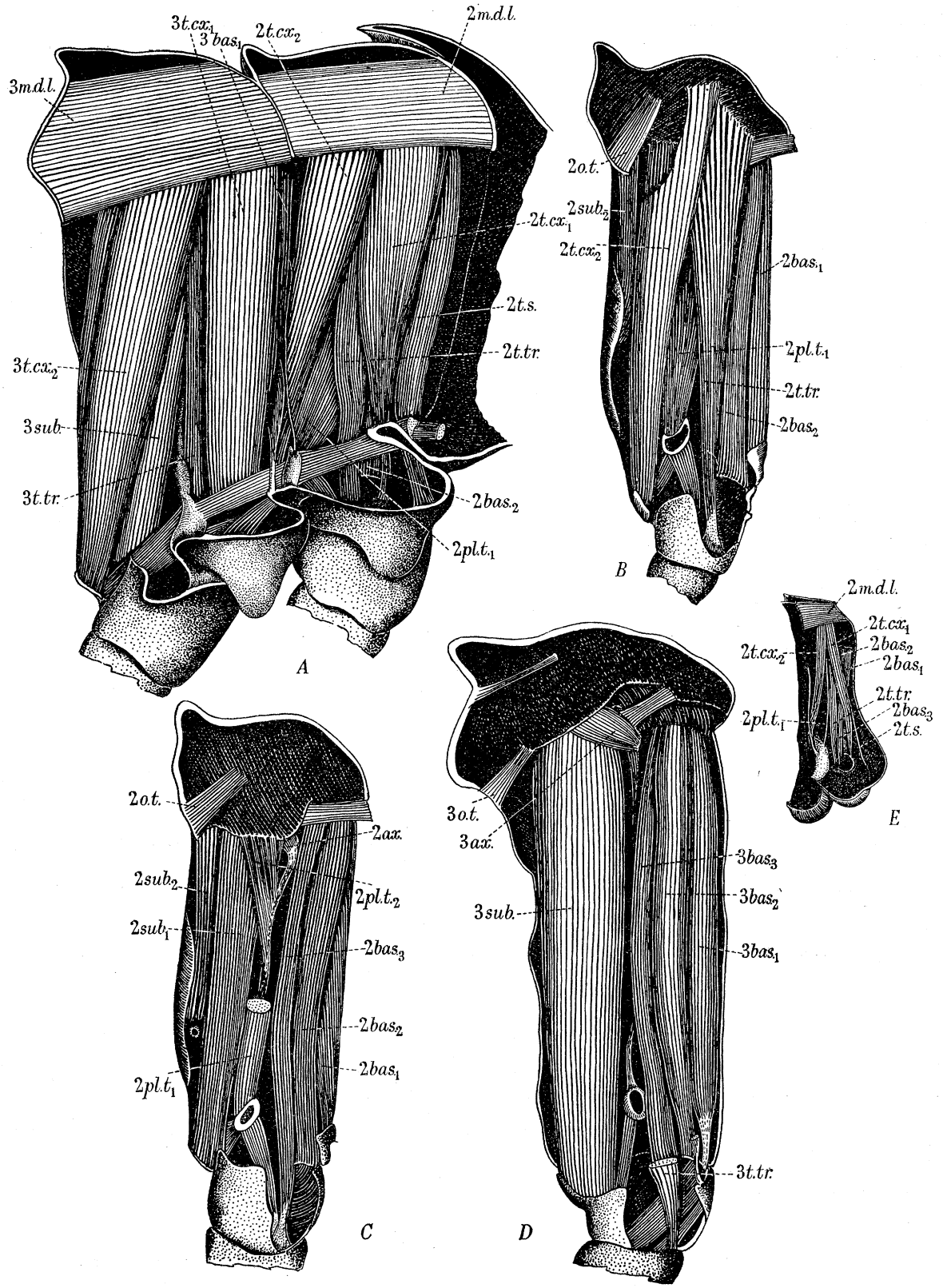
A. *Tergal muscles*

(i) *Median dorsal longitudinal muscle* (2 *m.d.l*) moderately well developed in male, but only very thin in female. In the latter the muscle has its hinder attachment on the antecosta of the metathorax, which is joined by flexible membrane to the mesothorax; in the male the chitinous phragma replaces the antecosta. A flexor of the thorax thus becomes, in the male, a wing depressor.

(ii) *Oblique tergal muscle* (2 *o.t*), a small muscle in both sexes, with hinder attachment as in (i), and with anterior attachment on scutum. It could, in the male, be a weak indirect wing depressor.

B. *Dorso-ventral muscles*

(iii) *Tergo-sternal muscle* (2 *t.s*), moderately developed in male, feeble in female. Its lower attachment is to the mesosternum, its upper to the prescutum lateral to (iv). In female it probably raises the tegmen by depressing the tergite; in male it is a weak wing levator.



IGURE 5. *Acridopeza reticulata.* *A*, median bisection of pterothorax; *B*, mesothorax, most medially placed muscles removed; *C*, mesothorax, lateral muscles exposed; *D*, metathorax, medial muscles removed; *E*, mesothorax of young nymph, drawn to scale.

(iv) *First tergo-coxal muscle* (2 $t.cx_1$), a fairly large muscle in male, but smaller in female; lower attachment by tendon to anterior rim of coxa; upper attachment near middle of scutum, with a thinner branch attached laterally on scutum just above the wing base. Apart from its promotor action on coxa, it could function in male as indirect wing levator.

(v) *Tergo-trochanteral muscle* (2 *t.tr*), with lower attachment to inner rim of trochanter, upper to thick wall of scutum lateral to (vi); of equal size in both sexes; could, apart from its depressor action on trochanter, function as indirect wing levator.

(vi) *Second tergo-coxal muscle* (2 $t.cx_2$), very large in male, much thinner in female. Lower attachment to sclerite in membrane at base of coxa, upper on a wide area of the scutum, and with a lateral branch above wing base (see figure 5*B*). Apart from its remotor action on coxa, it is one of the strongest wing levators.

C. *Pleuro-tergal muscle*

(vii) *First pleuro-tergal muscle* (2 $pl.t_1$), very large in male, thin in female; lower attachment to a prominent pleural apodeme, upper to scutum, lateral to (v); strong wing levator in male; raises tegmen in female.

D. *Pleural muscles*

(viii) *First basalar muscle* (2 bas_1), of moderate size in male, much thinner in female; lower attachment to trochantin of coxa, upper to basalar sclerite. Apart from its action on leg, it is in male a 'direct' wing depressor.

(ix) *Second basalar muscle* (2 bas_2), large in male, thin in female; lower attachment to outer rim of coxa, upper to basalar sclerite; abductor of coxa, and, in addition, in male, a 'direct' wing depressor.

(x) *Third basalar muscle* (2 bas_3) thicker in male; lower attachment by tendon to trochanter lateral to (v), upper to basalar sclerite; depressor of trochanter, and presumably 'direct' wing depressor in male.

(xi) *First subalar muscle* (2 sub_1), very large in male, thin in female; lower attachment to meron of coxa, upper to subalar sclerite. Remotor of coxa and, in addition in male one of the strongest flight muscles—'direct' depressor.

(xii) *Second subalar muscle* (2 sub_2), lower attachment on epimeron, upper on subalar sclerite; much larger in male; 'direct' wing depressor in male; depressor of tegmen in female.

E. *Wing adjustor muscles*

(xiii) *Second pleuro-tergal muscle* (2 $pl.t_2$), rather larger in male; lower attachment to pleural ridge, upper to scutum just above wing base. By drawing down the scutum it will, in male, tilt the wing up, or in female raise the tegmen.

(xiv) *Axillary muscle* (2 *ax*), a short muscle, arising from pleural ridge, and attached to third axillary sclerite; probably it rotates the costal margin upwards.

In the metathorax there are:

A. *Tergal muscles*

(i) *Median dorsal longitudinal muscle* (3 *m.d.l*), very large in male, delicate in female; function as in mesothorax.

(ii) *Oblique tergal muscle* (3 *o.t*), small in male, smaller still in female; possible indirect wing depressor in male.

B. *Dorso-ventral muscles*

(iii) *First tergo-coxal muscle* (3 $t.cx_1$), large in male, thin in female. In both sexes lower attachment is to membrane in front of base of coxa; promotor of leg, and, in addition, in male a powerful wing levator.

(iv) *Tergo-trochanteral muscle* (3 *t.tr*) of moderate size in male, thin in female. Lower attachment to inner rim of trochanter, upper to scutum. Depressor of trochanter, and, in male, an indirect wing levator.

(v) *Second tergo-coxal muscle* (3 $t.cx_2$), exceptionally large in male, thin in female. Lower attachment to sclerite in membrane at base of coxa, upper to scutum, with a smaller branch attached laterally on scutum. Draws back coxa, and is, besides, in male, one of the strongest wing levators.

There are no pleuro-tergals.

C. *Pleural muscles*

(vi) *First basalar muscle* (3 bas_1) of moderate size in male, weak in female; lower attachment to anterior rim of coxa, upper to basalar sclerite. Promotor of leg, and, in male, a 'direct' wing depressor.

(vii) *Second basalar muscle* (3 bas_2), large in male, thin in female; lower attachment to outer coxal rim, upper to basalar sclerite. Abductor of coxa, and, in male, a strong wing depressor.

(viii) *Third basalar muscle* (3 bas_3), medium size in male, thin in female; lower attachment to inner rim of trochanter lateral to (iv), upper to basalar sclerite; depressor of trochanter, and, in male, also a weak 'direct' wing depressor.

(ix) *Subalar muscle* (3 *sub*) exceptionally large in male, thin in female. Lower attachment to meron of coxa, upper to subalar sclerite. Remotor of leg, and in addition, in male, one of the strongest flight muscles ('direct' depressor).

D. *Wing adjustor muscle*

(x) *Axillary muscle* (3 *ax*), present in both sexes but larger in male, where it is attached to the third axillary sclerite.

(2) *Histology*

The muscle fibres are numerous and are usually disposed in bundles, and there is often a large trachea running internally along the muscle. Almost all the muscles are much thicker in the male, this being due both to an excess of muscle fibres, and to their individual enlargement. A cross-section of the first tergo-pleural of the male is shown in figure 122, plate 23; note the central trachea, and the disposition of the fibres in bundles. This is fairly general for the whole musculature.

The fibres are mostly polygonal in cross-section. The sarcolemma is a smooth membrane, best seen in the fresh tissue (figures 117, 118, plate 23). The abundant nuclei lie invariably just under the sarcolemma.

There are marked differences in the sarcostyles of the two sexes. In the male they measure a little under 1 μ thick, and there is no difficulty in displaying their component myofibrils; around the margin of the fibre they are lamellate. In the male they are thinner, and component myofibrils are recognizable only in the marginal sarcostyles, where the lamellate character is much less pronounced than in the female. The most

surprising differences relate to the fibril dimensions: in the female the inter-*Z* distance is as much as 9μ, reduced to 4·3μ, at full contraction; in the male the equivalent dimensions are 4·8 and 2·4μ respectively.

In the female the fresh fibre, cut in frozen section, shows a typical Cohnheim pattern, with an indication of lamellar pattern at the margin (figure 117). The male does not show a Cohnheim pattern (figure 118), and the fibril grouping, unlike that of the female, is very even. In the female there is a row of very minute *J*-granules to either side of the *Z*-membrane, united into a delicate reticulum. In the male the granules are bigger, being in places even large spherical sarcosomes up to 3μ in diameter (figure 118), but show no special cross-alinement, and tend to occupy only limited stretches of fibre.

Z-membranes, completely transecting the interfibrillar spaces, are readily seen; of the *M*-membranes I have been able to distinguish only the fibrillar (*Mf*) component. I have had no difficulty in recognizing the helicoidal disposition of the cross-membranes.

There is, in both sexes, a rich supply of tracheae, but denser in the male; and here alone they penetrate the sarcolemma (figure 118), though in small numbers only, and always they end blindly.

(3) *Development*

In the smallest nymphs that permit of dissection, all the muscles that are found in the adult can already be distinguished; the main difference is in the retarded development of those that are exclusively concerned with wing movement, and are therefore not functional in the nymph. Figure 5*E* represents such a dissection of the mesothorax, drawn to scale; specially noteworthy, as still rudimentary muscles, are the tergo-sternal and first pleuro-tergal, which, in comparison with the robust functioning muscles, are mere delicate threads. There is another such muscle, with intersegmental attachment (unlabelled); it is not a flight muscle, but survives in the adult as a vestigial muscle (shown but unlabelled in figure 5*A*), and is probably homologous with a functioning muscle in blattids and mantids (see figures 2, 3). The various pleural muscles all function as leg muscles in the nymph, but after the last moult acquire also a 'direct' action on the wings, when the epipleural sclerites separate from the pleurites (cf. Snodgrass 1929). The flexible junction between the tergites suggests that the dorsal longitudinals do not forego their primary role of flexors of the thorax till the phragmas begin to form in the last instar. Noteworthy also is a small change in lower attachment of the tergo-sternal muscle, from the membrane in front of the coxa on to the sternal wall.

(*a*) *Functional nymphal muscle*

I select, as an example, the mesothoracic first tergo-coxal. It is composed, in the adult, of 200 to 300 fibres, grouped in eighteen to twenty bundles. There are about the same number of fibres in both sexes, but in the female they are thinner.

In the minute first-instar larva the muscle is composed of only twenty fibres. A section is shown in figure 119, plate 23; the large transected object alongside the muscle is the muscle tendon; the spacious trachea of the adult muscle is represented by a thin vessel, indicated by *tr*.

In the early stages of nymphal growth the muscle enlarges purely by enlargement of its component fibres, which retain throughout a marginal zone of lamellae enclosing an axial

core of cylindrical sarcostyles; figure 120 shows such a fibre. But when the nymph has reached the length of about 1 cm, the entire muscle having enlarged to about twice its former thickness, fibre cleavage begins; in figure 120, for example, one of the fibres has split into three, and just below this, the two smaller fibres, judging by their size, are probably the product of an earlier cleavage. As in the other examples already given above, in which the fibres have a distinct sarcostyle pattern, the cleavage must break the pattern, but this is soon restored.

As the cleavage gains pace, it soon outstrips the rate of enlargement of the muscle, and in nymphs that are not more than 15 mm long, almost the full number of fibres has formed. Figure 121, plate 23, represents part of a section at this period of development. The fibres range considerably in size, some being less than 4μ across. In the larger fibres the normal sarcostyle pattern is evident, but this is not so in the smallest. Yet in rather later nymphs, where the fibres have again begun to enlarge, the normal sarcostyle pattern is restored.

In the female the pattern is retained even in the adult. In the male only a vestige of marginal lamellation survives, the lamellae disrupting largely into cylindrical sarcostyles.

In the final instar, fibre enlargement in the male outstrips that in the female. It is during this period that sarcosome aggregation proceeds, and in the male tracheae penetrate into the fibres.

Mitoses are found in considerable abundance in the muscle nuclei, particularly after fibre cleavage has become active. Close inspection shows that many are in the nuclei of tracheal cells. But it is plain that the muscle nuclei are also involved, though whether general for muscle nuclei is uncertain.

(b) Muscles with deferred function (pure wing-muscle)

I select for detailed description the first pleuro-tergal muscle of the mesothorax. It is an unusually large muscle, composed of over 800 fibres disposed in about twenty bundles, and with a central trachea running along the muscle (figure 122, plate 23).

We see the rudiment of this muscle already in the minute nymph (figure 5*E*), a fine bundle of very thin and imperfectly developed fibres, 4 to 9μ thick. Figure 123 shows a cross-section, taken from a 4 mm nymph; figure 124 is a longitudinal section from the opposite half of the same nymph. In both, a piece of normal functioning muscle is included for comparison. The cross-section shows about sixteen rudimentary fibres. The longitudinal section shows the abundance of muscle nuclei, compared with their paucity in the adjacent functioning fibre, and the absence of any cross-striation or even of cross-membranes. On close inspection the diminutive fibres are found to have each a sarcolemma, but this is not visible in the photograph.

In the growing nymph enlargement and cleavage of the rudimentary fibres takes place, but these rarely attain even a quarter the diameter of a functioning fibre. Cross-striations are now becoming evident; and as the fibres are very thin the helicoidal disposition of the cross-bands is easily recognized. Among the nuclei mitoses are abundant.

This cleavage proceeds into the penultimate nymphal instar, but the development lags behind that of functioning nymphal muscles. In the final instar the fibres thicken, acquire intracellular tracheae, and so become indistinguishable from the wing-muscles that have functioned in ordinary capacity in the nymph.

Other Tettigoniidae

For purposes of comparison, I have examined a few other members of this group.

The musculature I have dissected in the following: *Caedicia olivacea*, *C. valida*, *C. extenuata*, *Tinzeda eburneata*, *Xiphidium laetum*, *Paragryllacris combusta*, ?*Phyllophora* sp. In all, the arrangement of muscles is very similar to that of *Acridopeza*; particularly noteworthy are the single tergo-sternal and large pleuro-tergal of the mesothorax, the presence of a second subalar in that segment, and, in both segments, of a basalar operating on the trochanter. In *Tinzeda* the pure flight muscles (tergo-sternal and pleuro-tergal) are vestigial.

In *Caedicia*, as described earlier (p. 255), the muscles partake of the character of normal trunk muscle even more than in *Acridopeza*. A typical Cohnhein pattern is present (figure 94, plate 22), and compared with normal leg muscle (figure 44, plate 19) the only adaptations to flight are the presence of intracellular tracheae, and the more frequent occurrence of a considerable supply of sarcosomes.

In *Caedicia* and *Xiphidium*, where alone I have examined the tracheae, their penetration into the fibres has been found; but, consistent with their feeble power of flight, the intracellular vessels are not abundant. Mostly the vessels end blindly, though in *Xiphidium* I have seen clear instances of confluence between branches of adjacent tracheae. Among the extracellular vessels fusion is common.

The development I have examined is *Caedicia olivacea*; it takes place essentially as in *Acridopeza*.

ACRIDIIDAE

Chortoicetes terminifera Walker

This is the notorious Australian plague locust. Dr K. H. L. Key, from whom I have obtained my material, tells me that in a non-swarming population flight is normally limited to 10 to 15 yards; when swarming it is considerably more, but there is no sustained flight, for in a swarm advancing continuously the individuals are continually settling and rising again.

(1) *Myology* (figure 6)

Only such muscles as can conceivably function as flight muscles are described. These comprise in the mesothorax:

A. *Tergal muscles*

(i) *Median dorsal longitudinal muscle* (2 *m.d.l*), an unusually large muscle, attached to the phragmas; it is the principle wing depressor.

The oblique tergal is not present in this species.

B. *Dorso-ventral muscles*

(ii) *First tergo-sternal muscle* (2 $t.s_1$), a large muscle, attached above laterally on prescutum, below on sternum; wing levator.

(iii) *Second tergo-sternal muscle* (2 $t.s_2$), rather smaller than the former; upper attachment on scutum, behind and a little median to (ii); lower attachment on sternum in front of rim of coxa; indirect wing levator.

(iv) *First tergo-coxal muscle* (2 $t.cx_1$), attached above to scutum, and below to anterior rim of a coxa;part from a promotor action on coxa, it probably serves as indirect wing levator.

(v) *Tergo-trochanteral muscle* (2 *t.tr*), a large muscle arising by two branches from scutum; depressor of trochanter, and probably indirect wing levator.

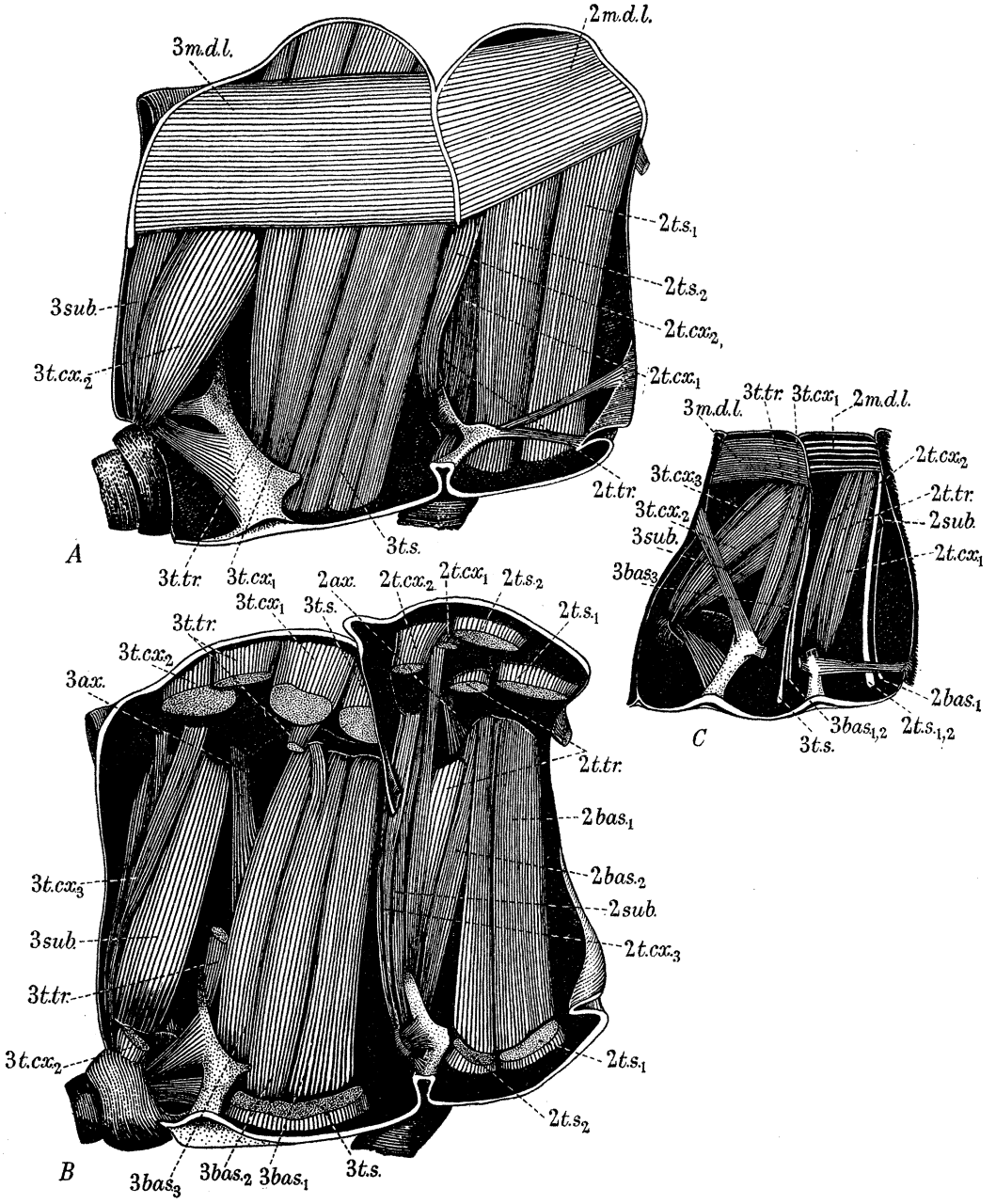


FIGURE 6. *Chortoicetes terminifera*. *A*, median bisection of pterothorax; *B*, medial muscles removed; *C*, first-instar nymph, not to scale.

(vi) *Second tergo-coxal muscle* (2 $t.cx_2$), a large muscle arising behind (iii) and attached below to hinder rim of coxa; remotor of coxa, and probably indirect wing levator.

(vii) *Third tergo-coxal muscle* (2 $t.cx_3$), a very thin muscle lying lateral to (vi), arising more laterally from scutum, and with long tendinous attachment to hinder coxal rim; remotor of coxa, and probably indirect wing levator.

C. *Pleural muscles*

(viii) *First basalar muscle* (2 bas_1), a very large muscle, attached below to sternum, lateral to area of attachment of the tergo-sternals; a powerful flying muscle, drawing the wing forward and downward.

(ix) *Second basalar muscle* (2 bas_2), a much smaller muscle lying behind (viii) and lateral to (v); upper attachment to basalar sclerite, lower to rim of coxa lateral to (iv); promotor of coxa, and probably 'direct' wing depressor.

(x) *Subalar muscle* (2 *sub*), a fairly large muscle immediately to the side of (vii); upper attachment to subalar sclerite, lower to membrane at base of coxa; abductor of leg, and probably 'direct' wing depressor.

D. *Wing adjustor muscle*

(xi) *Axillary muscle* (2 *ax*), arising from pleural ridge, and attached to third axillary sclerite.

The usual pleuro-tergal wing levator is not present.

The metathoracic muscles are even more strongly developed. They comprise:

A. *Tergal muscle*

(i) *Median dorsal longitudinal muscle* (3 *m.d.l*), a very large muscle, attached to the phragmas; it is the main indirect wing depressor.

There is no oblique muscle.

B. *Dorso-ventral muscles*

(ii) *Tergo-sternal muscle* (3 *t.s*), an exceptionally large muscle, attached above to prescutum, below to sternum. It is the main indirect wing levator.

(iii) *First tergo-coxal muscle* (3 $t.cx_1$), upper attachment to scutum, lower to anterior rim of coxa; promotor of coxa, and probably indirect wing levator.

(iv) *Tergo-trochanteral muscle* (3 *t.tr*), of medium size; inner branch attached behind (iii) to scutum, a lower thinner branch more laterally attached below (iii); lower attachment to trochanter. Depressor of trochanter and probably indirect wing levator.

(v) *Second tergo-coxal muscle* (3 $t.cx_2$), very large, upper attachment to scutum, lower to hinder rim of coxa. Apart from its powerful action on coxa used in springing, it could, with fixed coxa, act as indirect wing levator.

(vi) *Third tergo-coxal muscle* (3 $t.cx_3$), a thin muscle arising laterally on scutum, with tendinous attachment to hinder rim of coxa; remotor of coxa and possibly indirect wing levator.

C. *Pleural muscles*

(vii) *First basalar muscle* (3 bas_1), a strong muscle; upper attachment to first basalar sclerite, lower to sternum lateral to (ii); 'direct' wing muscle, drawing wing forward and downward.

(viii) *Second basalar muscle* (3 bas_2), immediately behind (vii), attached to both basalar sclerites; it lies close against (vii), but is actually a separate muscle.

(ix) *Third basalar muscle* (3 bas_3), behind (viii); upper attachment to second basalar sclerite, lower to membrane at base of coxa. Apart from an abductor action on leg, could act as 'direct' wing depressor.

(x) *Subalar muscle* (3 *sub*), a very large muscle, attached above to subalar sclerite, and below to hinder rim of coxa; apart from its remotor action on leg it is a powerful 'direct' wing depressor.

D. *Wing adjustor muscle*

(xi) *Axillary muscle* (3 *ax*), arising from pleural ridge, and attached to third axillary sclerite.

(2) *Histology*

The fibres are irregularly rounded in cross-section, measuring usually from 0·06 to 0·1 mm in diameter, though some reach 0·15 mm. The nuclei lie immediately under the sarcolemma. The sarcostyles are cylindrical, ranging in thickness from 0·5μ to nearly 1μ. They respond unusually well to fibrillar fixatives, from two to four component myofibrils being distinguishable in each sarcostyle. The inter-*Z* distance is 4μ (relaxed), reduced to nearly a half at contraction. The *Z*-membrane, marginally attached to the sarcolemma, is readily seen; of the *M*-membrane I have seen only the *Mf* component.

The muscle fibres are richly supplied with sarcosomes, but there is no evidence of a reticulum of interstitial substance delimiting muscle columns (figure 125, plate 23). The sarcosomes, which are spherical, are of considerable size (figure 125), and are arranged in transverse rows apparently at the level of each *Q*-band; they are, however, very easily displaced, both in isolated fresh fibres and in fixed preparations.

The tissue is richly supplied with tracheae, and these penetrate in large numbers into the interior of the fibres. The disposition of the intracellular tracheae is shown in figure 126, the photograph representing a fragment of the fresh tissue immersed in glycerine, the tracheae being sharply defined by their content of air. On close inspection with high-power objectives many of the tracheae are found to end freely, and their endings are probably genuine, for the enclosed air disappears only slowly in glycerine preparations. Tendency to closed net formation is, however, also apparent, branches from one tracheae merging directly with those of a neighbouring or opposite vessel. Close scrutiny of figure 126 will show several cases of this, even in the single plane of focus.

(3) *Development*

In the minute first-instar nymph all the main muscles of the adult can be distinguished, the pure flying muscles being, as usual, in a very rudimentary condition. These are more numerous than in other Orthoptera; in the metathorax there is the tergo-sternal and first and second (but not third) basalar muscles. In the mesothorax are the two tergo-sternals and first basalar. The median dorsal longitudinal of the mesothorax (but not metathorax) is also a pure flying muscle in *Chortoicetes*, for the nymph, unlike that of other orthopteran families, has no flexible articulation between the two hinder thoracic segments, so that the mesothoracic muscle does not function till the wings appear. The musculature of the first-instar nymph is shown in figure 6*C*; the labelling is the same as in figure 6*A*, *B*, but the drawing could not, owing to the small size of the nymph, be made to scale. Note in

the nymph the absence of 'phragmas' and the retarded development, particularly of the tergal region, where the tergo-sternal muscles are attached. Apart from this the only noteworthy skeletal changes are the separation of the two basalar and single subalar sclerites from the pleurites in the last nymphal instar.

(a) Functional nymphal muscles

Of these I select for detailed description the subalar muscle of the metathorax, a large compact muscle comprising, in the adult, between 500 and 600 fibres, that have a diameter range of 0·03 to 0·05 mm. There is no large axial trachea as in Tettigoniidae.

In the first-instar nymph the same muscle comprises only seventy to eighty fibres, cylindrical and very narrow, with an average diameter of 0·013 mm. The sarcostyles, in these very young fibres, appear evenly distributed in the cross-section, and are mostly cylindrical, but with a tendency to slight lamella formation around the fibre margin.

The usual fibre enlargement takes place during the early growth period of the nymph, the marginal lamellae becoming more pronounced. In the third penultimate instar, when the fibres have attained an average width of about 0·025 mm, cleavage begins. It affects an increasing number of fibres, which cleave, equally or unequally, usually into two or three daughter fibres. The rate of cleavage soon outstrips that of enlargement of the muscle, so that early in the penultimate instar a five to six-fold increase of fibres has taken place, though the area of cross-section of the entire muscle has increased to only about three times its initial size. The average diameter of the fibres is thereby reduced to about 0·01 mm, and some are no more than 0·004 mm in width. The striking change that is thus brought about is shown by figures 127 and 128, plate 24. Figure 127 is from a nymph in the third penultimate instar, and shows the fibres at their maximum size, though here and there cleavage is beginning; figure 128 is a similar section from a penultimate instar nymph, in which about 400 daughter fibres are already present. Mitoses among the muscle nuclei are abundant.

In the last instar the muscle, whose growth has, till now, merely kept pace with that of the nymph, accelerates its development in preparation for the new function of flight, the muscle enlarging up to three or four times its diameter at the inception of the instar, while the nymph enlarges by less than twice. The sarcosomes now also appear in quantity, and tracheae penetrate the sarcolemma.

The above description applies to all functional muscles, though in some of them the onset of cleavage is much retarded.

(b) Muscles with deferred function (pure wing-muscle)

The dorsal longitudinal of the mesothorax will serve as example. It is a very large muscle, comprising up to 1300 fibres, disposed in six indistinctly delimited bundles. This large muscle takes its origin from six rudimentary muscle fibres that comprise the imperfectly developed dorsal longitudinal muscle of the mesothorax. Their disposition is shown in figure 129, plate 24 (indicated by asterisk), which represents, at low magnification, a cross-section of the left upper quarter of the mesothorax of a first-instar nymph; the thin-walled heart, at the top of the section, will serve as convenient landmark. A fragment of the section at high magnification is given in figure 130, and shows two of

the six rudimentary fibres; a longitudinal section is given in figure 131. Particularly noteworthy is the rich content of closely packed nuclei, the presence of only a few fibrils, but complete absence of cross-striation.

In the early nymph fibre enlargment proceeds only very slowly. A cross-section from the third penultimate instar is shown in figure 132. The contents of the fibre have begun to split into subcolumns of fibrils, but these are still enclosed in a common sarcolemma. There is frequent mitosis among the nuclei; cross-striations are still only doubtfully present.

As in other Orthoptera the cleavage rate soon begins to outstrip that of enlargement. Early in the penultimate instar about half the definitive number of fibres has already appeared, but they are surprisingly thin, averaging only about 5μ in width, and some being no more than half this. Most of the diminutive fibres have acquired a sarcolemma, the entire progeny of a single parent fibre apparently being still ensheathed in the original membrane. Mitosis continues actively, the fibres being surprisingly rich in nuclei. Striations are now present, at least in places, but are still very faint. Figure 133, plate 24, shows a section through one of the six fibre bundles, from a late penultimate instar nymph, in which considerable enlargement of the muscle rudiment, beyond the stage already described, has taken place.

The intersegmental phragmas are now beginning to form, the daughter muscle-fibres, terminally attached by tonofibrillae to the epidermis, being thereby carried to their definitive position in the thorax.

The final stages present nothing unusual, and do not merit detailed description. There is the usual fibre growth, the striation becoming more distinct; sarcosomes accumulate in abundance, and penetration of the tracheae into the muscle fibres takes place.

(*c*) *The muscle attachment* (*tonofibrillae*)

Chortoicetes will serve as a convenient type on which to describe the development of the muscle insertion. In the first-instar nymph the rudimentary fibre from which the dorsal longitudinal muscle will form is connected with the epidermis, and its individual fibrils with individual tonofibrillae of the epidermal cells. With enlargement of the nymph the zone of attachment grows, slowly at first, but much accelerated in the final instar as the phragmas develop. The cells that are involved are, in contrast to those of the surrounding epidermis, long and slender (figure 134, plate 24), the tonofibrillae forming a compact bundle in each cell. At the margin of the zone, the basement membrane of the surrounding epidermis merges with the sarcolemma of the muscle fibre (figure 134). Expansion of the area of attachment involves the recruitment of cells from the surrounding epidermis. Surprisingly, within the latter, tonofibrillae are already present, and are apparently directed towards the muscle fibre by the continuous sarcolemma-basement membrane sheath. But it is plain that the cleavage of already attached cells is also taking place. The evidence lies in the presence of mitoses in such cells; in figure 135, for example, the prophase nucleus (asterisk) is plainly that of a cell in the middle of the zone of attachment and already has tonofibrillae. When eventually the chitin of the phragmas is secreted, the formerly slender epidermal cells flatten out against it.

Other Acridiidae

Many members of this group can move at considerable speed through the air, and are certainly more accomplished fliers than are the other Orthoptera. Nevertheless flight, even at its best, is crude, and with little ability to steer in the air. The well-attested flight of migratory locusts far from land is commonly attributed to air currents; yet I have seen locusts crossing the Red Sea, aided by only the slightest breeze.

I have dissected the following: *Acrida conica*, *Austroicetes vulgaris*, *Locusta migratoria*, *Goniacea australasiae*, *Coryphistes* sp., *Austracris guttulosa*, *Monistria roseipennis*. Data for *Dissosteira* are available from Snodgrass (1929). There is a general uniformity in the plan of the musculature: the median dorsal longitudinal muscles are always strongly developed, and all the specific wing muscles described above for *Chortoicetes* are found here also. These comprise, in all cases, the two tergo-sternals in the mesothorax, and the single large tergo-sternal of the metathorax, together with the basalar muscles that have, as in *Chortoicetes*, lost their association with the leg, and acquired a new attachment to the sternum. Noteworthy also is the absence of any basalar muscle operating on the trochanter. In the metathorax the hinder basalar shows a tendency to divide into two, and in *Goniacea* consists of two completely separate muscles.

Of exceptional interest is *Monistria roseipennis*, where the female is wingless, and only some of the males winged. Even in the wingless forms all the muscles that have been described above in *Chortoicetes* are recognizable, but are all much smaller than in the winged members; and, as in the case of *Acridopeza* among the Tettigoniidae, this may be taken as good evidence that they all exercise, in addition to their action on the leg, a secondary function in flight. The specific flight muscles are exceptional, being, in the wingless members, reduced to mere delicate wisps. Thomas (1953) has recently described such vestigial muscles in other Acridiidae with reduced wings.

The histology of the muscle fibres on the whole resembles that of *Chortoicetes*. In all cases there are intracellular tracheae; in the rather weakly flying *Austroicetes* they are less abundant, and there is little evidence of closed net formation. In the wingless *Monistria* there are no intracellular tracheae. Sarcosomes are always abundant, even in wingless *Monistria*, and lie in transverse rows.

I have obtained a large series of nymphs of another acridiid, *Acrida conica*; development is as in *Chortoicetes*, and does not need description.

General remarks on Orthoptera

In the foregoing, representatives of all the families of Orthoptera except Grylloblattellidae have been examined, so that the description is probably valid for the whole order. The tergal muscles, functional in one capacity in the nymph, undergo change of function by development of phragmas in the last instar, and so become wing depressors. In this new role they co-operate with the epipleurals, which in the adult acquire the ability to pull directly on the wing base, and are sometimes (mantis, mole cricket) even the principal wing depressors. The dorso-ventrals, in turn, are all potential flying muscles, for with fixation of the leg they must depress the tergal wall. To what extent individual epipleural

and dorso-ventral muscles do exercise this double function has not been directly observed; the evidence from *Acridopeza* indicates that they are extensively used in this way, for in the male they are nearly all much larger than in the flightless female, and have, moreover, the distinctive histology of the flying muscles, which the female lacks. *Acridopeza* shows, moreover, that this secondary action on the wings can be achieved even without change in muscle insertion, for no essential difference has been discovered in this respect between the sexes.

The Orthoptera thus give a surprisingly intelligible picture of the early adaptation of the thoracic muscles to the function of flight. This should not, however, blind us to the fact that certain muscles of the nymph and even of the wingless species, namely, the pleurals, seem to have no counterpart in the muscles of *Apterygota*, but are adaptations to a pterothorax; Snodgrass (1935) has already drawn attention to the fact that the pleurites of the pterothorax, to which these muscles are attached, are skeletal adaptations to flight.

The impression is given, when we survey the lower families of Orthoptera, that depression of the wing was initially produced by the pleural muscles attached to the epipleural sclerites (epipleural muscles); certainly in the metathorax of blattids, of mantids and of gryllotalpas, the median tergal muscles are very ineffective in this role, while in the mesothorax of blattids and mantids it is the oblique muscles that are called into service. Only in gryllids and grasshoppers do the median longitudinal muscles acquire importance, becoming in the Acridiidae the main wing depressors.

Whether the tergo-sternal (pure flying) muscles have any forerunners in flightless insects is not clear. A single such muscle is usually present in the mesothorax of lower Orthoptera, and may be a coxal muscle that has changed its attachment; but the additional tergo-sternals of Acridiidae are surely new muscles.

The musculature of the Acridiidae merits special comment. Here flight and the development of a specific wing musculature both reach their highest level. Specially noteworthy is the presence of several large tergo-sternals, a change in attachment of one of the basalars on to the sternum to become a pure flying muscle, and the detachment of the third basalar from the trochanter. It might be suspected that with the evolution of this specific wing musculature, other dorso-ventral muscles temporarily pressed into service of flight would revert to pure leg muscles. Judging by their histology this is not so; even such unlikely muscles as the tergo-trochanteral, or the coxal remotor (springing muscle) of the third leg, show the rich intracellular tracheation characteristic of wing muscle (the prothoracic leg muscles, for comparison, lack intracellular tracheae). It is noteworthy that in Acridiidae the pleuro-tergal (flying muscle) of Tettigoniidae is not present.

Turning next to the histology of the muscular tissue, a progressive structural adaptation of the fibres to the demands of flight is at once evident. In *Blattella* and *Periplaneta* the fibres are indistinguishable from normal leg muscle, though in another blattid (*Panesthia*) tracheae penetrate into the fibres in considerable numbers. Among Tettigoniidae, *Caedicia* has fibres that still display typical Cohnheim areas, the only features distinguishing them from its own leg muscle being an increased sarcosome content, and penetration of some tracheae into the muscle fibres. Yet in *Acridopeza* the fibres in the male are already markedly different from those of the female. Finally, in Acridiidae there is an abundant sarcosome content, and without Cohnheim pattern, and there is a rich intracellular tracheation with

a tendency to closed net formation, bringing with it the possibility of flow of air through the fibre. Here the Acridiidae approach the higher insects.

Throughout the Orthoptera, growth of the thoracic (including prothoracic) muscles has been found to arise partly by fibre thickening, but mainly by fibre proliferation, in which latter respect it differs from that of *Ctenolepisma*. The cleavage does not impair visibly the functioning of the muscle. The final histological specialization of the wing muscle tissue has been found, as expected, to take place during the last nymphal instar. Of exceptional interest are the pure wing muscles; these have no function in the nymph, and their structural differentiation is therefore much retarded. They can, however, be recognized even in the minute nymph as very delicate rudimentary muscle fibres. They seem to provide the clue to the remarkable condition in cicadas (description, p. 299), where the entire bulky wing musculature arises out of a few such rudimentary fibres.

I cannot give any new facts on the innervation. At present the only adequate description is that of Mangold (1905) on *Decticus*; the nerve terminals ramify within a 'Doyère eminence', just under the sarcolemma, and two separate nerve fibres, that undergo parallel branching within the muscle, are said to end together in the same motor end-organ.

HOMOPTERA

Evidence so far obtained indicates that the Homoptera hold a position of special significance in the evolution of the flying mechanism. None of the members are really accomplished fliers, and indeed most of the metathoracic musculature is usually diverted to the function of springing. But in the histology of the flying muscles there is specialization beyond anything found in Orthoptera, even leading in some groups to the production of coarsely fibrillated Siebold muscle. The histogenesis also differs widely, even in related groups, suggesting a plasticity that has led to the evolution of the type of histogenesis found in higher insects. Finally, there are most remarkable differences in the character of the musculature: in one group (cicades) the utilization of leg muscles solely for the purpose of flight has reached the limits of expediency; in others, again, the musculature approaches that of the higher insects.

The following description covers only certain groups of Homoptera; the Heteroptera have not been examined.

CICADIDAE

Cyclochila australasiae Donovan

This is a large green cicada, about 4 cm long (including wings), very plentiful in south-eastern Australia, where its shrill song is one of the most familiar forest sounds in the early summer. The newly emerged nymph is a white sluggish ungainly creature, about 2 mm in length, that soon drops to the ground from the trees in which the eggs are laid, then burrows into the soil, where it sucks the juices from the roots, attaining maturity after a period that is believed to be about 4 or 5 years. The nymphs live underground mostly at a depth of 1 or 2 ft., and the collecting of an adequate number of developmental stages is therefore a laborious task; it would indeed have been impracticable but for the fact that

the insect has acquired a predilection for introduced European oaks, along the roots of which the nymphs tend to congregate.

Most of its short life the adult spends resting in the foliage of trees, though when the need arises it can fly tolerably well. Held in front of a stroboscope a wing beat of 25/s is recorded.

(1) *Myology* (figure 7)

Mesothoracic muscles

A. *Tergal muscles*

These are powerfully developed and comprise the chief flight muscles. Their hinder attachments are to an exceedingly large mesothoracic phragma, laterally attached and curving forwards, almost along the floor of the segment, as far as the second leg.

(i) *Median dorsal longitudinal muscle* (2 *m.d.l*), a very large muscle running forward from the phragma, and attached in front to the arching wall of the thorax. Its fibres are disposed into five bundles; wing depressor.

(ii) *Oblique tergal muscle* (2 *o.t*), a very large muscle attached below to the phragma, and above along the hinder medial half of the scutum. Its more medially placed fibres run an almost vertical course to their insertion on the scutum, but the lateral fibres veer into an oblique position (cf. figure 7*B*). It is the principal wing levator.

Snodgrass (1927) has drawn attention to the remarkable condition of the 'oblique' muscle in cicadas, which by the forward growth of the phragma is drawn into an almost dorso-ventral position, to become the antagonist of the longitudinal muscle. It is probable that these two tergal muscles act also on the second wing, for there are no tergal flight muscles in the metathorax, its tergal wall being reduced to a mere vestige.

B. *Dorso-ventral muscles*

(iii) *Tergo-sternal muscle* (2 *t.s*), a fairly large muscle, with upper attachment to prescutum, lower on basisternum; wing levator.

(iv) *Tergo-coxal muscle* (2 *t.cx*), a thin muscle, upper attachment just lateral to (iii); lower attachment on anterior rim of coxa.

(v) *Tergo-trochanteral muscle* (2 *t.tr*), a thin muscle arising lateral to (iv); lower attachment by tendon to inner margin of trochanter, the tendon being shared by fibres arising within the coxa itself, and by a muscle that arises from the pleural apodeme (depressor of trochanter, 2 *d.tr.*).

The condition of (iv) and (v) is very remarkable. They are plainly homologous with the first tergo-coxal and tergo-trochanteral of Orthoptera. But they develop only during the nymphal period, and in their histology are not leg muscle at all, but typical cicada wing muscle (description below). From their delayed appearance, and from their histological character, we hesitate to assign them any action on the leg, and regard them as pure flight muscles (wing levators and extensors). The second tergo-coxal of Orthoptera is also represented in the cicada; but it is a pure leg muscle (2 *cx.rm.*).

C. *Pleural muscles*

(vi) *First basalar muscle* (2 bas_1), rather small, arising from basalar sclerite, and attached to basisternum lateral to (iii).

(vii) *Second basalar muscle* (2 bas_2), just behind (vi), and having its lower attachment to a small sclerite in membrane at base of coxa.

(viii) *Third basalar muscle* (2 bas_3), attached to trochanter by a tendon in common with (v).

(ix) *Subalar muscle* (2 *sub*), a short thick muscle, attached above to subalar sclerite, and below to meron of coxa.

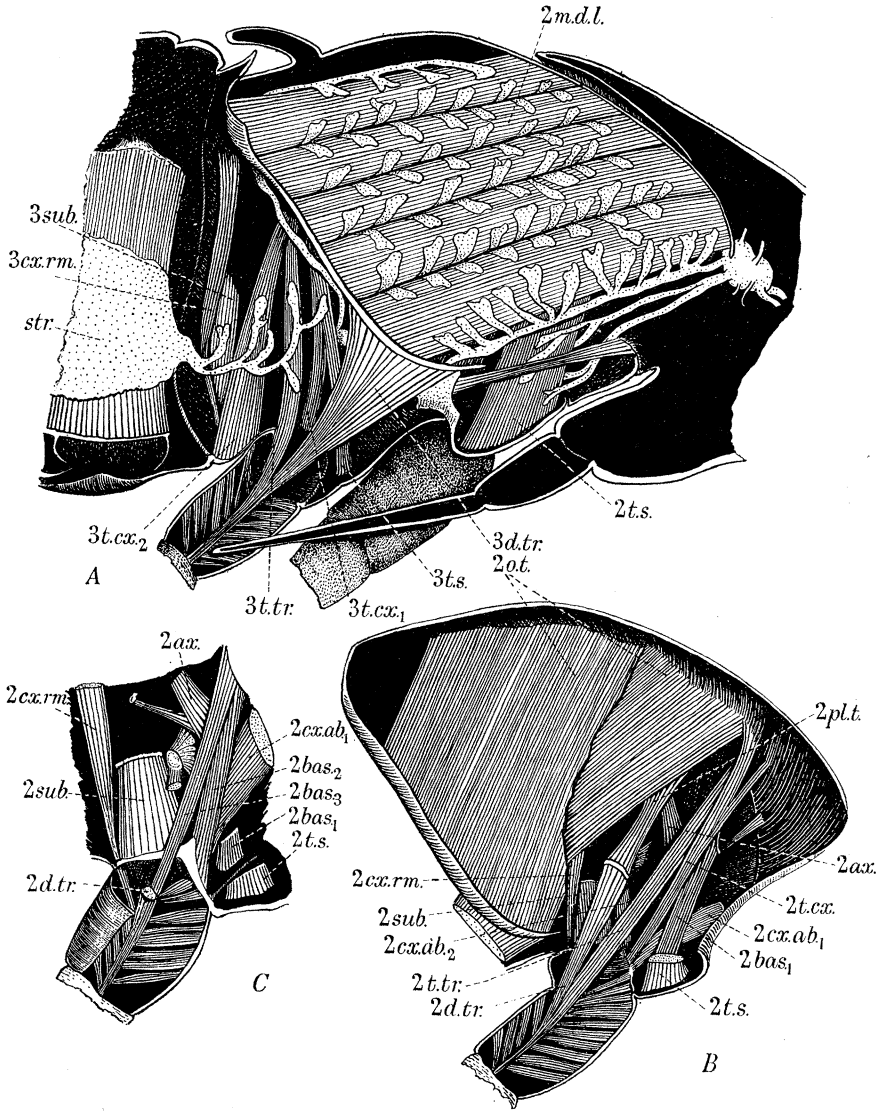


FIGURE 7. *Cyclochila australasiae.* *A*, median view of muscles; *B*, median muscles removed (mesothorax only); *C*, muscles associated with base of second leg, exposed by removal of median muscles. Included in the drawing are certain muscles, referred to in the text, that are not wing muscles, viz. $cx.ab_{1,2}$, first and second coxal abductors; *cx.rm*, coxal remotor; *d.tr*, depressor of trochanter; *str*, stridulating muscle of abdomen.

(*Note on notation.* In this, and subsequent dissections, the pure wing muscles are given a morphological notation, while the muscles that operate on the leg are designated functionally; e.g. the tergo-trochanteral muscle, operating as pure wing muscle, is designated *t.tr*; the same muscle, acting exclusively as depressor of trochanter, is designated *d.tr*, as are also any other muscles with similar function.)

All these muscles are plainly homologous with similarly named muscles of Orthoptera; but they all arise during the nymphal period, and histologically are wing muscle. For these reasons we regard them as pure flight muscles (wing depressors).

D. *Wing adjustor muscles*

(x) *Pleuro-tergal muscle* (2 *pl.t*), attached just above the wing base; as in Orthoptera it tilts wing up.

(xi) *Axillary muscle* (2 *ax*), attached to third axillary sclerite.

Metathoracic muscles

Apart from a few almost vestigial longitudinals, there are no tergal muscles, and certainly none that function in flight.

A. *Dorso-ventral muscles*

(i) *Tergo-sternal muscle* (3 *t.s*), a weak muscle arising laterally from the very reduced tergal wall, just above the wing insertion; wing levator.

(ii) *First tergo-coxal muscle* (3 $t.cx_1$), a thin muscle attached to anterior rim of coxa; wing levator.

(iii) *Tergo-trochanteral muscle* (3 *t.tr*), rather larger than the former. It operates on trochanter by a tendon in common with a much larger depressor (3 *d.tr*), arising from phragma, but which has, of course, no function in flight. Wing levator.

(iv) *Second tergo-coxal muscle* (3 $t.cx_2$), attached to meron of coxa. Wing levator.

B. *Pleural muscle*

Basalars are absent; the only muscle in this group is:

(v) *Subalar muscle* (3 *sub*), lying just lateral to (iv). Wing depressor.

Muscles (i) to (v) are histologically of wing muscle type, and arise during the nymphal period.

It is evident from the foregoing account that the cicada exemplifies a truly remarkable stage in the evolution of wing musculature. Apart from the surprising specialization, whereby the two tergal muscles of the mesothorax are made to function, in antagonistic capacity, as the principal wing vibrators, the general plan of the musculature is very reminiscent of that of lower Orthoptera. But in Orthoptera the pleural and dorso-ventral muscles associated with the legs are all functional limb muscles in the nymph, accepting the additional role of wing muscles after the last moult; in the cicada, on the other hand, the nymph dispenses with them, and they mature in the last nymphal instar to become flight muscles. Whether, in the adult, they exercise their potential action on the leg is uncertain; leg movement in the adult is sluggish, as in the nymph, and, as their histology suggests, they are probably purely concerned with flight.

In dissections of young nymphs the various muscles that act on the leg are readily seen. They survive, enlarged, in the adult, but of course lack the special histology of the flight muscles. Those of the mesothorax have been incorporated in figure 7; they are: (i) a (first) coxal abductor (2 $cx.ab._1$) arising from the episternum; (ii) a coxal remotor (2 *cx.rm*), arising from the scutum, both muscles being present in Orthoptera, and where the latter is

a wing muscle; (iii) a depressor of the trochanter (2 *d.tr*) arising from the pleural apodeme; (iv) a (second) coxal abductor (2 $cx.ab_2$), with similar origin; (v) a coxal adductor, also arising from the apodeme, but not included in the drawing.

Tracheal supply (figure 7*A*). In this, and subsequent types, the tracheae are described, because of the intimate association of their development with that of the wing musculature.

The principal trachea of the pterothorax is a rather thin vessel that passes forward from the metathoracic spiracle along the lower margin of the dorsal longitudinal muscle, to the first spiracle, located in the membrane between the first two segments. From it several branches pass up over the lateral surface of the longitudinal muscle. There is also a major tracheal vessel (shown in figure 7*A*) passing up from the metathoracic spiracle just behind the phragmas, which it penetrates above, to supply a large branch to the upper part of the thorax.

These tracheae do not break up directly into branches to the muscle fibres, but expand into numerous air-sacs that are directly applied to the outer surfaces of the muscle, and from which the branches to the muscle fibres then arise. Air-sacs are very conspicuous also on various other muscles, e.g. those in the head.

The metathoracic muscles derive their tracheal supply from the above-described vessel that passes up behind the phragmas, and from a large air-sac that invests the stridulating muscle.

(2) *Histology*

(*a*) *Muscle fibre*

The flying muscles present a quite unusual histology, which they share with the large stridulating muscle, and which seems indeed to be specific for Cicadidae.

Within a muscle the fibres are grouped into bundles, bound together by tracheae. From seven to twenty fibres compose a bundle, and they also are held by tracheae. Each bundle is enclosed in a membranous sheath containing nuclei. The sheaths are shown in figure 9*E*.

The fibres traverse the whole length of the muscle, and in the case of large muscles are from 6 to 8 mm in length. They do not display any constant form, being sometimes cylindrical, more commonly polyhedral. Fibre thickness ranges from 0·06 to 0·09 mm.

The sarcolemma is, as usual, best seen in the fresh fibre (figure 136, plate 24). The nuclei are flat disks, lying invariably just under the sarcolemma.

Cross-sections show a complexity of sarcostyle pattern that is specific for cicada muscle. Figure 136 represents a fresh cross-section; finer detail can be made out only on the fixed preparation (figure 137). The general picture given by the fresh and fixed preparation is one in which the sarcostyles are disposed in complex lamellae which, often with some branching, radiate from the middle of the fibre. In the fixed tissue the sarcostyles show a special grouping within the lamellae, seen in cross-sections mostly as rings; the fresh fibre can readily be interpreted in terms of the fixed preparation. The appearance of the longitudinal section will depend on its orientation with respect to the lamellae; in figure 138, for example, the lower half of the photograph is composed of sections that pass through the lamellae, while in the upper half it grazes along a lamella.

The sarcostyles number about two thousand for a fibre of medium size. They are less than 1 μ in diameter (fixed preparation), and as already described above in the general

account of sarcostyles, contain three or four myofibrils that are bound together by the ground substance of the sarcostyle. The inter-*Z* distance in the relaxed sarcostyle is about 3μ, which, at full contraction, is reduced to about two-thirds.

In connexion with the cross-membranes we have to consider the special question as to whether they completely transect the fibre, including the interlamellar spaces, or whether they are confined to the lamellae. It must at once be observed that in the fresh fibre there is a perfect transverse alinement of cross-striations, and this suggests the presence, also, of complete transverse membranes. But certainly most preparations give no hint of a complete membrane, even with good *Z*-membrane staining. But subsequent experience has shown that discontinuity of the membrane across the interlamellar spaces is due to rupture in the process of fixation, the spaces, as in figure 138, plate 24, being considerably widened in the preparation of the tissue. Usually the stained preparation shows only the *Zf*-disks, without interfibrillar connexion, and *Mf* is completely unstained, giving indeed the illusion of a clear line across the fibre, as in figure 138. In material fixed with trichloracetic acid, a good presentation of the *Z*-membrane has appeared (figure 149), but without any indication of *M*. Unexpectedly the most exquisite staining of the cross-membranes has come from material fixed in Carnoy, and stained for nerve by Willis's silver method. Photographs are given in figures 147 and 148. In figure 147, judging by the spacing of the fibrils, the section passes along a lamella; in figure 148 it passes, on the left, along a lamella, but elsewhere it transects the lamellae, the fibrils being grouped, and separated by wider interlamellar spaces. Observe in both photographs the sharply defined *M*-membrane completely transecting the interfibrillar spaces between successive *Z*-membranes; and observe, in figure 148, how both *Z* and *M* cross the interlamellar spaces.

The sarcoplasm occupies the spaces within and between the lamellae, and is best seen in cross-sections of the fresh fibre (figure 136). There is a rich content of fairly large sarcosomes, and these are transversely alined (figure 139). Within the lamellae they are quite regularly disposed, there being a double row to each cross-striation; in the interlamellar spaces this double alinement is also seen in places, but in other places is conspicuously absent, possibly in consequence of disorganization through fixation.

Since the fibres are thick and easily distorted by manipulation, the pattern of fibril organization is necessarily difficult to see. Yet in thick sections of fixed fibres, where there seemed no reason to suspect distortion, helicoidal effects have been readily apparent. In young fibres, before the final enlargement takes place, the effects are more easily seen (figure 165, plate 25).

(b) Tracheae

There is a moderate supply of intracellular tracheae, which arise from thin branching vessels that emerge from the above-described air-sacs. These grow along the surface of the fibre, penetrate the sarcolemma, and as Kölliker (1888) long ago found, are confined to the interlamellar spaces (they can be seen, for example, in the cross-sectioned fresh fibre shown in figure 136). The tracheae therefore adopt a distinct pattern within the fibre, which we have not met in any of the forms so far described.

The tracheae stain unusually well with the Da Fano method, the picture of the longitudinal section depending on its orientation with respect to the lamellae. Where the

section transects the interlamellar spaces we see the cut tracheae alined in rows, and find a complete absence of tracheae within the lamellae (figure 143). But where the section passes along an interlamellar space, the full expanse of the branching tracheae is seen, almost without adjusting the focal plane. Figure 142 shows such a view of the tracheae. The two figures are complementary, and will enable the reader to form a picture of the organization of the internal air tubes. None of the branches seem to end blindly, and sometimes even without change of focus, confluence between branches of adjacent vessels is seen.

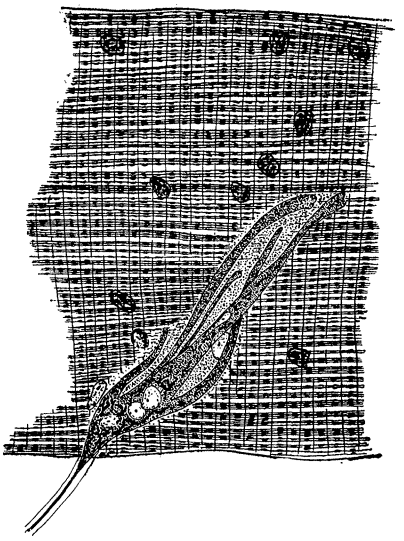


FIGURE 8. Motor nerve ending on muscle fibre of *Cyclochila australasiae*, (gold chloride).

(*c*) *Innervation*

I have had considerable success in staining the intramuscular nerves with the conventional gold chloride method. In bulk stained material, spread out in glycerine under a cover-glass, the branching nerves can be followed for long distances among the muscle fibres, on which they terminate by motor end-organs. Close inspection shows the nerves to be encased in a nucleated sheath, but the enclosed nerve fibres are not well presented by this method.

Although the Da Fano process brings out both the nerve sheath and its contained nerves, much the most effective preparations have been obtained by Willis's silver method. Here it is at once evident that the nerve sheath, at best only faintly stained, and usually not at all, encloses two separate nerve fibres, one thick and one thin, and that the twin fibres undergo simultaneous branching within the nerve sheath. Figure 141 shows a fragment from the thicker part of the nerve trunk, figure 140 from a region of medium size. In the former, in which alone the nerve sheath is dimly outlined, the contrast between thick and thin fibre is especially evident. I have been able to follow the twin fibres almost to the motor end-organ. The delicate fibre is by this time reduced almost to invisibility; indeed, it is usually recognizable only by a few beaded varicosities. I infer that it enters the motor end-organ, but on this final point the preparations so far obtained fail.

Figure 150, plate 25, is a photograph of a motor ending, as shown by gold chloride. More structural detail is given in figure 8; the drawing shows the branching fronds of the

thick motor fibre (the thin is, of course, not visible), embedded in a local aggregation of granular protoplasm, containing a few nuclei. In cross-sections of silver preparations the hypolemmal position of the motor ending is evident (figure 137, plate 24); in all cases I have found the nerve to be confined to the zone immediately below the sarcolemma, i.e. it does not pass between the fibrils.

(3) *Development*

This begins in the very young nymph that measures not more than 3 or 4 mm in length. I have had much difficulty in obtaining an adequate supply of these small underground creatures, and can therefore give only a bare outline of the initial phases of development. These will be given with reference to the tergo-sternal muscle, the earliest rudiments of which are more easily identified in sections. All the subsequent stages are more conveniently described with reference to the large dorsal longitudinal muscle. It will be understood that the general character of the development is the same for all the flying muscles and for the stridulating muscle, but not, of course, for the functioning nymphal muscles (the latter multiply by ordinary cleavage, as in Orthoptera, and do not require description).

The dorsal longitudinal muscle consists of some 1500 fibres, disposed into about 120 fibre bundles, and itself comprises five separate but closely adjoined muscles (figure 9*E*). This very large muscle arises from a small group of about a dozen myoblasts which in the newly emerged nymph lie clumped midway along the principal tracheae of the meso-thorax, just under the roof of the segment. I have not been able to identify the myoblasts from which the other muscles arise.

The next developmental stage that I have obtained is from a 3 mm nymph. cut in cross-sections. It is shown in figure 151, plate 25, and represents the first recognizable rudiment of the tergo-sternal muscle (about a third its length). A group of myoblasts has here united into a column, the cells having lost their individuality; but within the column no structural differentiation is yet visible. A recognizably later developmental phase is shown in figure 152; about six fibrils have now appeared (not all visible at single focus), and as they have a distinctly granular texture we may suspect that they have arisen from some granule precursor of the cytoplasm. In several nymphs from 4 to 5 mm long, perceptible cross-striation has already appeared in the fibrils, and it is indeed evident that a delicate rudimentary muscle fibre has now formed. By a process that is, basically, a repeated longitudinal fibre cleavage, the entire muscle will arise out of this rudimentary fibre. It will be convenient to describe these events with reference to the median dorsal longitudinal muscle.

Initially the rudimentary fibre undergoes much enlargement, its fibrils increasing in number and its nuclei multiplying, but there is no attendant cleavage into daughter fibres. In the 1 cm nymph the rudiment has enlarged to a degree where it can readily be seen, even with low magnification, immediately below the roof of the thorax (figure 9*A*). A high-power view is shown in figure 153, plate 25; a longitudinal section from the opposite half of the same nymph is shown in figure 154. A very thin investing sheath (sarcolemma) is visible, and we are particularly struck by the abundance and crowding of nuclei. In this particular specimen I cannot see cross-striation, but this is certainly present in another nymph of the same size, though exceedingly faint.

Cleavage of this enlarged but still rudimentary muscle fibre into five separate fibres now takes place. This phase is shown in figure 155; it constitutes the first cleavage phase and is a critical stage in development of the longitudinal muscle, for from the five fibres the five component muscles will arise.

The events now to be described take place in nymphs ranging from 10 to 15 mm in length; they constitute the second cleavage phase, and result eventually in a splitting of

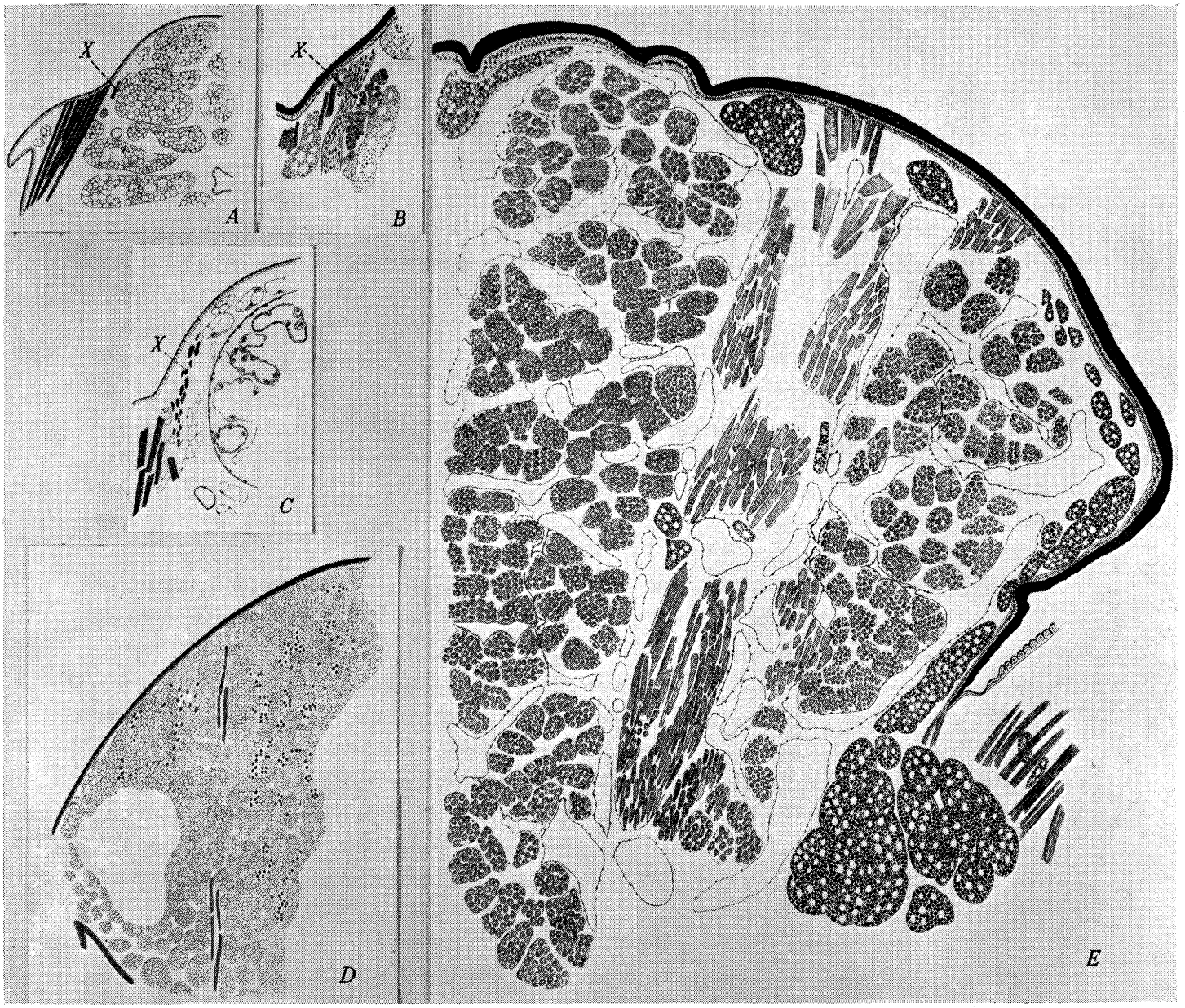


FIGURE 9. Cross-sections of mesothorax of five nymphs at successive stages of development, and drawn to scale, showing growth of dorsal longitudinal wing muscle. *A*, 10 mm nymph; *B*, 12 mm nymph; *C*, 15 mm nymph; *D*, 20 mm nymph; *E*, advanced nymph. In *A*, *B* and *C* the muscle rudiment is indicated by *X*, the labelling line in *C* pointing to the particular fragment that is shown at higher magnification in figure 157, plate 25. In *D* the rudiment has spread down into the fat-body; in *E* it is the massive wedge-shaped structure to the left. Fragments of the oblique wing muscle appear, unlabelled, in *B*, *D* and *E*.

the five fibres each into a group of exceedingly delicate, still rudimentary fibres numbering over a hundred, of which each is the forerunner of one of the above-described fibre bundles. Initially there is some enlargement of the five parent fibres, but this is soon followed by some cleavage. The latter now begins to outstrip the rate of enlargement, and so the products of cleavage begin to diminish in size. Figure 156 represents a fragment at this stage of development, and though the fibrils are fewer, it recalls the type of cleavage described above for Orthoptera. At the end of this cleavage phase the fibres are reduced to minute dimensions, most of them ranging from 3 to 6μ in diameter. A lower power view of the cross-section is given in figure 9*C*, the small fragment to which the labelling line points being shown at high magnification in figure 157. Within these minute fibres, the fibrillae lie mostly marginally. Longitudinal sections (figure 158) show actually more clearly than the cross-section, that there is an axial column of sarcoplasm, within which the elongate nuclei lie; close scrutiny of figure 158 also shows that the fibrils are faintly cross-striated, having both *Z*- and *Q*-bands.

We pass now to the final stage of development (third cleavage phase), in which the delicate tubular fibres become converted into the fibre bundles. This takes place in the next (penultimate) instar. The thoracic wall is now beginning to assume final shape, and the phragmas are in process of forming. The minute muscle fibres, attached at their ends to the epidermis, thereby become drawn down into their definitive position within the thoracic cavity, which is, at this period, mostly occupied by fat-body. Through the fat-body the delicate fibres run, either singly or in very fine bundles (figure 9*D*), and here they undergo the final stages of their development. Initially there is the usual fibre enlargement, but in this case it is attended from the beginning by the appearance of most delicate internal partitioning walls, within which single fibrils, or at most two or three such fibrils, become encased (figure 159, plate 25). Here, then, we see yet another cleavage process; it is the final (third) phase of cleavage, and it brings about the formation of the definitive muscle fibres. A slightly later stage is shown in figure 160; enlargement has begun again, and the fibril content of the daughter fibres is increasing, presumably by splitting of fibrils. This is still more marked in figure 161, in which also the first indication of separation of daughter fibres is evident. In longitudinal sections the fibres now consistently show well-marked cross-striations, but without any tendency to helicoid formations (figure 162).

As the fibril proliferation proceeds, an elegant rosette pattern makes its appearance in the cross-section (figure 163), and as this becomes better defined (figure 164) we recognize the developing lamellar pattern of the adult fibre.

The final stage takes place in the last nymphal instar. This is marked by much enlargement of the fibres, and by an increasing complexity of sarcostyle pattern as the lamellae branch and even generate new lamellae; the muscle helicoid is now readily seen (figure 165). When the fibril proliferation is at last completed, the concluding phase of enlargement of fibrils into sarcostyles sets in, and is attended by an increase in their spacing, and therefore by a general thickening of the fibre.

A point in regard to the cross-membranes merits comment. We have seen above that in the adult fibre the cross-membranes bridge the interlamellar spaces. From the manner of formation of the lamellae it at once follows that *Ms* and *Zs* must actively grow across these spaces, and that they do not arise passively by a drawing apart of cleavage fibrils.

The sarcosomes are detectable only in the final phase of development, and do not seem to arise from any microscopically visible cell precursor.

As stated above, the fibre bundles are themselves enclosed in a nucleated sheath. With fine needles it can be separated from the fibres when the muscle is hardened in alcohol, and it is plainly not a tracheal net. It arises actually from the sheath that encloses the developing fibre bundle, and is evidently a derivative of the sarcolemma of the parent fibre from which the fibre bundle arose. It is seen in figures 9*E* and 163, plate 25. The nuclei are a residue not incorporated into the daughter fibres.

Although tracheae penetrate the developing fibre bundles quite early (a few are visible in figure 163), it is only late in their development that the muscle fibres themselves acquire their supply. In fibres in which the sarcostyle pattern is already established, a considerable meshwork of tracheae is forming around the fibres. They impregnate unusually well with the Da Fano process, and cross-sections of the prepared tissue soon begin to show the tracheae growing into the fibres along the interlamellar spaces; in figure 146, for example, there is already extensive penetration of such vessels into the muscle fibres. From the very beginning branching of the entering vessels occurs, and if sections are taken along the interlamellar spaces, closed net formation is soon evident. In figure 144, for example, it seems to be starting in some of the tracheae, while in the older fibre shown in figure 145, blindly ending tracheae are no longer seen. Only after the last moult do the tracheae fill with air.

The development of the zone of attachment is essentially as in *Chortoicetes* described above, and does not need description.

Other Cicadas

(1) *Myology*

Cyclochila is a member of the highest subfamily Cicadinae. For comparison I have examined several species of Tibicininae (*Melampsalta torrida*, *Cystosoma saundersi* ('bladder cicada'), *Diemeniana richesi*, *Pauropsalta encaustica*). In all cases the musculature is as in *Cyclochila*.

(2) *Histology*

Throughout the group there is also a remarkable uniformity in the structure of the muscle fibres. All display the very distinctive histology above described for *Cyclochila*; this statement is based on the following material: Tettigarctinae: *Tettigarcta crinita*; Tibicininae: *Melampsalta torrida*, *M. abdominis*, *Pauropsalta encaustica*, *P. leurensis*, *Abricta aurata*, *Diemeniana richesi*, *Cystosoma saundersi*, *C. schmelzi*; Cicadinae: *Psaltoda moerens*, *Macrotristria angularis*, *M. hillieri*, *M. intersecta*, *Arunta perulata*. The descriptions given by Ciaccio (1887) for *Cicada plebeja*, and by Kölliker (1888) for *Tibicina hematodes*, conform in general with the above description.

(3) *Development*

For comparison with *Cyclochila*, I have obtained a large series of nymphs of *Pauropsalta encaustica* Germer to serve as representative of the lower group of Tibicininae. It is a small black cicada that inhabits the mountainous forest country of eastern Australia, and is very

abundant in summer. The nymphs suck the sap from the surface roots of eucalypts. My material ranges from 3 mm nymphs up to fairly late stages, though the final phases are lacking.

In the 3 mm nymphs the rudiments of the future wing muscles are already present, in the form of delicate imperfectly developed muscle fibres with faint cross-striation, much as in *Cyclochila*. The familiar fibre enlargement, with proliferation of nuclei and of fibrils, now ensues, but the cross-striation remains faint, and is, indeed, often unrecognizable.

Cleavage then follows. Its general character is shown in figures 166 to 169, plate 26, which represent cross-sections of the left upper half of the mesothorax, at the level of the tergo-sternal muscle. The upper end of this muscle is shown in each figure on the left; on the right, some distance above the main trachea, is the dorsal longitudinal muscle. In figure 166 (6 mm nymph), cleavage of the tergo-sternal muscle is in progress; the dorsal longitudinal muscle rudiment has completed its first cleavage into five separate fibres, which are themselves entering the second cleavage phase. In figure 167 (from the same instar, but approaching ecdysis) the second cleavage phase, with general enlargement of the rudiment, is proceeding, and in figure 168 is about complete.

Continuing to enlarge, the rudiment spreads down into the thoracic cavity. Figure 169 shows a cross-section from a 1 cm nymph, but at smaller magnification. The third cleavage phase is here about complete, the definitive muscle fibres, grouped in fibre bundles, having appeared. This third cleavage evidently takes place earlier in *Pauropsalta* than in *Cyclochila*, where it is delayed till after the fat-body has insinuated itself between the developing fibres.

The final phase of fibre enlargement, during which the 'rosette' pattern of the cross-section appears, takes place, as in *Cyclochila*, with the fibres completely immersed in invading fat-body, into which they have been drawn by downgrowth of the phragma.

JASSIDAE

Erythroneura ix Myers

This is a small green 'leafhopper', measuring about 3 mm in length (excluding wings), that lives and breeds often in plague numbers, on grass, clover and various weeds. It is a comparatively active insect, hopping when disturbed, and readily taking to the wing; but it is not a strong flier, and seeks shelter after a flight of only a few yards. The wing frequency is 29 to 31/s (stroboscope determination).

(1) *Myology* (figure 10)

Mesothoracic muscles

A. *Tergal muscles*

(i) *Median dorsal longitudinal muscle* (2 *m.d.l*), attached behind to the large forwardly curving phragma, and in front to the arching wall of the thorax. It is the main wing depressor.

(ii) *Oblique tergal muscle* (2 *o.t*), an upwardly directed muscle, arising from the phragma, and attached above laterally on scutum. Wing levator.

We have here the same arrangement of muscles as in cicadas, whereby two tergal muscles, acting antagonistically, become the principal wing vibrators.

B. *Dorso-ventral muscles*

(iii) *Tergo-sternal muscle* (2 *t.s*), a comparatively large muscle; upper attachment on scutum, lower on basisternum. Wing levator.

(iv) *Tergo-coxal muscle* (2 *t.cx*), lying immediately behind (iii); its lower attachment is partly on sternum and partly on membrane at base of coxa, and is probably a former leg muscle that has undergone slight change of attachment (in cicadas it is actually attached to rim of coxa). Wing levator.

These are the only flight muscles in the dorso-ventral group, alone having the histology of flight muscles, and alone arising during the nymphal period. The others are exclusive leg muscles, functional even in the nymph, and comprise: (*a*) a thin tergo-coxal muscle (2 *cx.pr*) acting by a long tendon on the anterior rim of the coxa (coxal promotor); (*b*) a thin tergo-trochanteral muscle (2 $d.tr_1$), which is a depressor of the trochanter, to which it is attached by a tendon that it has in common with a pleural muscle (2 $d.tr_2$), and with fibres arising within the coxa (in cicadas this muscle is actually a pure flying muscle); (*c*) a second tergo-coxal (2 *cx.rm.*), acting by a tendon on the coxa (coxal remotor).

C. *Pleural muscles*

(v) *Basalar muscle* (2 *bas*), attached above to basalar sclerite, and below to basisternum lateral to (iii). 'Direct' wing depressor.

(vi) *Subalar muscle* (2 *sub*), a small muscle, operating on subalar sclerite by a short tendon. 'Direct' wing depressor.

The presence of only a single basalar wing muscle is noteworthy. Included in the pleural group is a coxal abductor (2 *cx.ab.*), lateral to (v) operating on anterior rim of coxa (present in Orthoptera).

D. *Wing adjustors*

(vii) *Pleuro-tergal muscle* (2 *pl.t*), unusually long. Tilts wing up.

(viii) *Axillary muscles*, two triangular muscles, lying lateral to the subalar, and attached by tendons to third axillary sclerite, one at its anterior, the other at its posterior end. The hinder muscle probably tilts the costal margin up; the front probably reverses this action (not shown in figure 10).

Metathoracic muscles

The flight muscles are here weak; the tergal wall is relatively much larger than in cicadas, but this is to provide attachment for the large springing muscle.

A. *Tergal muscles*

(i) *Oblique tergal muscle* (3 *o.t*), a rather short muscle, apparently a wing depressor. Note the absence of a median dorsal longitudinal muscle.

B. *Dorso-ventral muscles*

(ii) *Tergo-sternal muscle* (3 *t.s*), a weak muscle, attached below to basisternum; wing levator.

(iii) *Tergo-coxal muscle* (3 *t.cx*), a weak muscle that passes obliquely across the segment, and is attached below to the membrane above hinder rim of coxa; wing levator.

There are three other muscles in this group, but they are not flight muscles: (*a*) *coxal promotor* (3 *cx.pr*), lying lateral to (ii); (*b*) a coxal remotor (3 *cx.rm*); (*c*) a powerful springing muscle (3 *spr*), actually the tergo-trochanteral of the group, occupying most of the metathorax and operating on the trochanter by a short powerful tendon.

C. *Pleural muscle*

(iv) *Subalar muscle* (3 *sub*), lateral to (iii); direct wing depressor.
This is, as in cicadas, the only epipleural muscle.

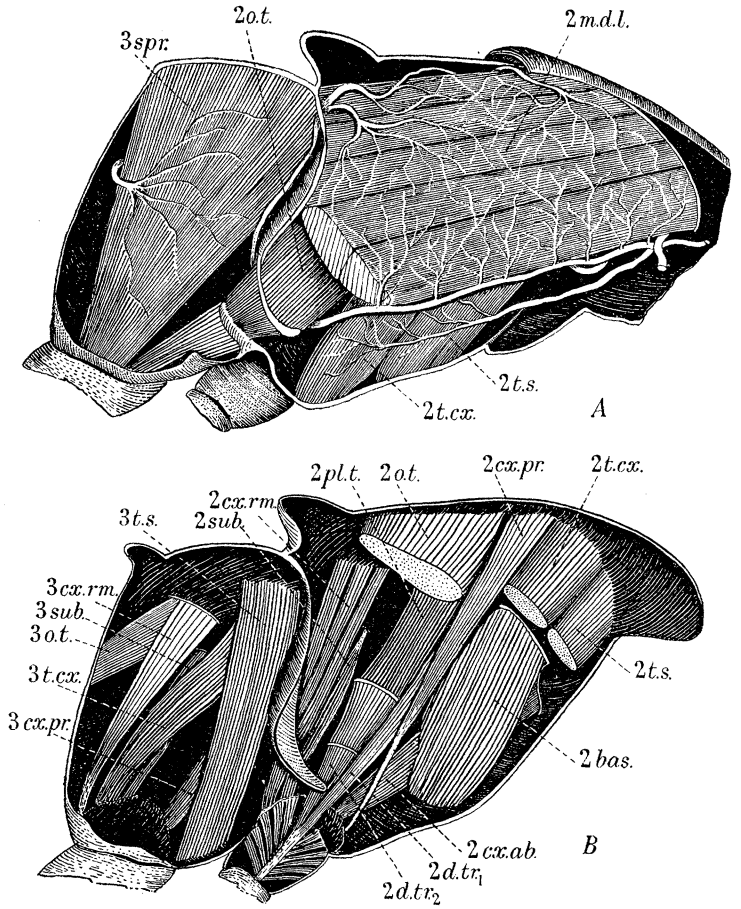


FIGURE 10. *Erythroneura ix.* *A*, median bisection of pterothorax; *B*, dissection, showing lateral muscles.
For notation see footnote to figure 7, p. 294.

Tracheae

The character of the tracheal supply can be seen from figure 10*A*, which shows such of the tracheae as are visible in a median bisection. The main mesothoracic trachea passes forward from the metathoracic to mesothoracic spiracle, along the lower surface of the median dorsal longitudinal muscle, which it supplies with numerous branches that spread out on the surface of the muscle. The upper and lateral portions of this muscle derive their supply largely from a big vessel that passes up behind the phragma before it enters the mesothorax (visible in figure 10*A*). The more laterally placed muscles also are supplied by this vessel, and by a tracheae that passes back from the first spiracle.

A. *Wing vibrators*

(2) *Histology*

(*a*) *Muscle fibre.* In the wing vibrators the muscle fibres are unusually large, measuring in some cases up to 0·07 mm in width, so that individual muscles contain only a few fibres each; e.g. the mesothoracic median dorsal longitudinal consists of five such fibres (figure 171, plate 26), the oblique tergal of three, the tergo-sternal, tergo-coxal and basalar muscles of one each. In the metathorax the thin tergo-sternal muscle consists of two fibres, but they are exceptionally narrow (0·022 to 0·03 mm).

TABLE 1

fibre from	no. of nuclei	no. of sarcostyles
median dorsal longitudinal muscle	804	712
	851	725
	568	627
	553	547
tergo-sternal muscle (mesothorax)	1159	961
	740	719
	877	814
tergo-sternal muscle (metathorax)	193	179
	263	259
	358	389

In cross-section the fibres are mostly polygonal or square. A sacrolemma is present, but can be consistently shown only in transverse sections (figure 181, plate 26). In longitudinal sections it is often confused with a marginal sarcostyle; when clearly visible it shows the usual festooning at its periodic attachments to the *Z*-membranes.

The nuclei, which are very abundant, are oval and lengthwise disposed. About half of them lie immediately below the sarcolemma; the others are scattered, usually in longitudinal rows, among the sarcostyles, which they exceed a little in diameter. In the thin fibres of the metathorax most of the nuclei lie just below the sarcolemma.

From the character of the histogenesis, described below, there are grounds for suspecting a numerical equality between the nuclei of a fibre and its sarcostyles. That a rough relationship does exist is indicated in table 1. The data for nuclei have been obtained from complete serial cross-sections of selected fibres. The sarcostyle counts are exact; the nuclear counts are necessarily subject to some small error. The sarcostyles are of the coarse Siebold type, measuring about 2μ (fresh) in thickness, except in the weak tergo-sternal muscle of the metathorax, where they range from $1{\cdot}5\mu$ to less than 1μ (figure 92, plate 21). Figure 181, plate 26, shows the coarse sarcostyles as seen in a Da Fano preparation; figure 192, plate 27, is from a conventional preparation, of indifferent quality, but of interest because it gives an instance of sarcostyle cleavage (marked by asterisk), the cleavage extending from the insertion on the chitin up to the thirteenth *Z*-membrane. In appropriately fixed material six to eight myofibrils can be seen in the coarse sarcostyles; in the thinner sarcostyles of the metathoracic muscles only three or four are present.

Of the cross-membranes *Zf* is visible in the fresh tissue, and even in inferior preparations (e.g. figure 192), and a *Zs* connexion across the intersarcostyle spaces is commonly seen. Of the *M*-membrane I have seen only the *Mf*-component. The inter-*Z* distance is 3μ.

There is necessarily some difficulty in examining the disposition of the cross bands—i.e. whether helicoidal or otherwise—because of the tendency of the fibres to dissociate into sarcostyles. I find that fibres fixed *in situ* and cut in thick sections do show typical helicoidal effects if examined near their insertion on to the chitin, where fibril displacement is necessarily reduced to a minimum.

There is a rich supply of sarcosomes, which are transversely alined in a double row to each cross-band.

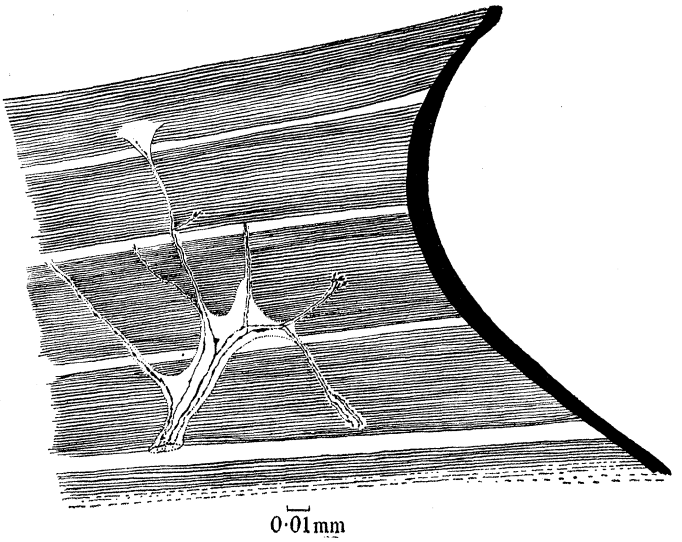


FIGURE 11. Drawing of a section along the dorsal longitudinal muscle of *Erythroneura ix*, showing innervation. The drawing represents approximately the hinder third of the muscle (phragma to right), to which the innervation is confined. From a Da Fano preparation. The drawing has been much simplified by omission of all stained tracheae. The axon-impregnation is more complete in the lower than in the upper half of the drawing. The three severed fibres are passing to the oblique muscle.

(*b*) *Tracheae.* A fibre of average size is supplied along its length by twenty to thirty separate small tracheae. Often the tracheal end-cells lie below the sarcolemma (figure 183, plate 26), in other cases outside it. The fine tracheae, devoid of spiril intima, run mainly lengthwise among the sarcostyles (figure 182); many end blindly, while others merge with branches of adjacent vessels to form a completely closed net. The tracheal supply, though ample, is not rich; thus in cross-sections of well-impregnated Da Fano preparations, we find about forty to fifty transected tracheae in a fibre having some 600 sarcostyles.

Since the sarcostyles are evenly distributed in the fibre, it is surprising to find evidence of a tracheal pattern within the fibre, the entering tracheae tending to expand in planes. If, for example, in figure 182 the upper end of the fibre to the right is inspected, the tendency of the tracheae to enter the section in a row is at once evident. The development of the fibre, described below, will show how this is brought about.

(*c*) *Innervation.* The nerves impregnate remarkably well with the Da Fano process, and since the insect is small and the muscle fibres few, an unusually clear picture of the innervation is obtained. The dorsal longitudinal muscle, which alone I have examined, is supplied by a single large nerve trunk that grows up along the lateral surface of the muscle in the hinder third of the segment (figure 11), and branches to supply each muscle fibre

(and also the oblique muscle) with a single motor end-organ. The sheath of the nerve ('neurilemma') merges with the sarcolemma of the muscle fibre, being sometimes (figure 193, plate 27), but not always (figure 181), raised into a conspicuous 'Doyère eminence'. Within the main nerve up to six fine nerve fibres can be distinguished, but the details of their distribution are difficult to make out. In the best preparations there is no doubt whatever of the presence of two separate nerve fibres in the final branches that go to form the motor end-organ, and these can, moreover, be traced into the end-organ itself (figure 193). In favourable places the twin fibres can even be seen to arise from two separate fibres of the nerve trunk; but in most instances the impregnation does not give a clear picture on this point. It is noteworthy that the twin fibres are not, in this species, of markedly different thickness.

B. *Wing adjustor muscle*

In comparison with the wing vibrators it will be useful to have data on at least one insect, and the present species will conveniently serve the purpose.

In the pleuro-tergal muscle of the mesothorax six or seven fibres are present. They measure about 0·015 mm across, and are, of course, not of the coarse fibrillar type. There are thirty-five to forty nuclei per fibre, lying just below the sarcolemma. Sarcosomes are present in considerable number, but are not alined. The tracheal supply is poor, and there are no intracellular vessels. There is a double nerve supply, the twin fibres branching together to supply two fibres to the motor end-organ (figure 172, plate 26).

(3) *Development*

A. *Wing vibrators*

It will be convenient to describe the myogenesis with reference to the mesothoracic dorsal longitudinal muscle; in general, of course, the description applies to all the flight muscles. The leg muscles, with which we are not directly concerned, enlarge by growth of their fibres, but without fibre cleavage.

The longitudinal muscle takes it origin, not from a pre-existing nymphal fibre, as in the foregoing types, but from a small number of free myoblasts. For most of the muscles the myoblasts can be only doubtfully identified in the minute nymph; those of the dorsal longitudinal muscle are exceptional, for they lie in a clear space below the tergal wall, immediately above the main trachea of the segment. They are short spindly cells, lengthwise disposed, with densely granular cytoplasm, and in the smallest nymphs that I have obtained (0·7 mm long) number not more than four (figure 175, plate 26).

Soon they increase in number, to form a packed mass of cells that fills the enlarging space between trachea and body wall. Then, in nymphs measuring only 0·9 mm, and with about thirty myoblasts present, the myogenesis proper begins. Several of the myoblasts (eventually five), becoming increasingly filamentous, grow along the column of myoblasts for the full length of the segment, and at their ends fuse with the intersegmental epidermis. Each is the pioneer myoblast that initiates the development of one of the five muscle fibres. The cytoplasmic granules of the myoblast do not remain with the nucleus, but spread along its tenuous prolongations, where they then fuse into a continuous fibril. Figure 176 represents a section along the muscle rudiment at this initial phase of develop-

ment; included in the section are a fragment of the trachea (to right), six myoblasts (one heavily granular) and a single pioneer myoblast which by focus adjustment* has been drawn for its entire length, and within which the single fibril is visible.

The pioneer myoblast thus contributes three structural components to the future muscle fibre—a single fibril, an investing film of cytoplasm (sarcoplasm), and a single nucleus. It remains to examine the part that the free myoblasts now play in completing its construction. Among the myoblasts cell proliferation becomes more active, and soon they form a swarming mass around the muscle rudiment (e.g. figure 180, plate 26). While this is taking place, the fibrils of the rudimentary fibre are plainly increasing in number, and are even beginning to show cross-striations (figures 178 to 180); the muscle nuclei too, though they evince no evidence of division, are becoming more abundant. As the myogenesis proceeds it becomes indeed more and more evident that the free myoblasts are in some way becoming incorporated into the growing fibre, for there is no further heaping up of myoblasts, even though mitosis among them continues unabated. Then, as the mitosis subsides, the myoblasts gradually decrease in quantity, and in the advanced nymph are no longer found.

The process of myoblast incorporation can be followed only in longitudinal sections; but cross-sections are at least helpful, for they show more effectively how the myoblasts swarm around the developing fibre. Figure 187 represents such a cross-section from a very young nymph, and shows the fine developing fibres encased in a swarm of free myoblasts (the reader will gain a better impression of the location of the muscle rudiment by referring to figure 186, in which the asterisk shows it in position in the transected thorax).

In these young nymphs a complete succession of developmental stages can be found, in which the fibrils progressively increase in number. Figure 177 shows the two-fibril stage (from the oblique muscle), figure 178 the three-fibril stage. In figure 179, seven fibrils are present, and in the transected fibres shown in figure 187, the fibril range is from ten to twenty.

In the two-fibril stage, which has been drawn in figure 177 with full attention to minute detail, it is evident that a second myoblast has added itself to the pioneer myoblast, and has brought with it a second fibril not yet complete at one end, where its granule precursors are still clumped (there are three free myoblasts in the section; the cells at the end of the fibre rudiment are, of course, epidermal cells).

The completed three-fibril stage is shown in figure 178; the presence of a third muscle nucleus indicates the incorporation of a third myoblast (the giant cell is an oenocyte; the reader should not readily confuse the three muscle nuclei, marked with asterisk, with the granule-laden myoblasts). These are also shown in figure 189, which is part of another fibre also at the three-fibril stage of development.

The seven-fibril stage is shown in figure 179; of the expected seven nuclei six are seen in the clump of cytoplasm midway along the fibre; several myoblasts are in mitosis.

Gradually, in this way, the fibre is built up to its full dimensions, and therewith the last of the myoblasts has vanished. But direct observation of the actual incorporation of the myoblasts has proved a matter of the utmost difficulty; it is, indeed, most disconcerting

* To take full advantage of focus adjustment, this and several succeeding stages are shown by drawings in preference to photographs.

to know that multitudes of myoblasts are being built into the growing fibre by a process that almost evades detection even in the best preparations. This arises partly from the fact that, especially in the later phases of development, the crowding myoblasts almost obscure the field of vision at the surface of the fibre where events are proceeding. Only in exceptional preparations, and where the field of vision permits, do we get even a hint as to how it is achieved. In such preparations we see, lying along the surface of the developing fibre, delicate fibrils, devoid of cross-striations, sometimes running a rather wavy course; and in favourable places these can be seen to arise as delicate filamentous outgrowths from myoblasts that have become applied to the surface of the fibre (figure 180). But only in the rarest cases is it possible to follow them for any distance before they become obscured.

The picture of the myogenesis that seems to emerge is the following: here and there a free myoblast becomes applied to the surface of the developing fibre, and, in the manner of the pioneer myoblast, undergoes bipolar elongation. Fusing with the growing fibre, it adds to the latter a single nucleus, a single fibril, and some additional sarcoplasm. The approximate numerical equality of nuclei and sarcostyles in the adult fibre suggests that a single myoblast actually extends the full length of the growing fibre, and nucleus-fibril counts of growing fibres support this belief; e.g. in the five growing fibres shown in figure 187, the number of nuclei (counted in serial cross-sections) is 18, 18, 22, 18, 10, the fibril number being 18, 17, 20, 19, 10 respectively; in the more advanced fibre shown in figure 188 the nuclei number 86, 114, 121, 102, 53, and the fibrils 80, 103, 118, 101, 56 respectively. These counts, which are subject to only small error, are very suggestive; but complete histological demonstration is not possible.

A point that emerges from the fixed preparations is that the fibrils are, from the very beginning, subject to organization within the growing fibre, for the cross-striations of the fibrils are transversely alined (figures 179, 180). To this pattern the newly incorporated fibrils accommodate themselves, and so build up the cross-striation of the whole fibre. With not more than twenty fibrils present, I have seen clear cases of helicoidal configuration. The demonstration of cross-membranes, which presumably impose the organization, is very capricious in the young fibre; I have seen evidence of it as early as the seven-fibril stage.

A question that at once obtrudes is that of the sarcolemma: is its presence compatible with progressive myoblast incorporation? Suitable preparations can leave no doubt that the membrane is indeed present, even before myoblast incorporation has ended. This is at once seen in figure 190, plate 27, where the sarcolemma is readily identified by its continuity with the basement membrane of the epidermis at the zone of attachment (cf. figure 134 for *Chortoicetes*). In these young fibres the nuclei lie in the marginal accumulation of sarcoplasm, and this is wholly invested in sarcolemma; but in cross-sections this bulky sarcoplasm is found to extend only incompletely around the developing fibre, leaving the fibril column partly exposed (this is well seen in figure 188, second fibre from left). Here, also, the sarcolemma is absent, and here, presumably, myoblast incorporation takes place. Figure 190 shows that the sarcolemma is not yet 'festooned'; it vindicates also the view that the sarcolemma is a true plasma membrane, and not a product of the tracheae (see Introduction, §A).

From their initial position at the margin of the fibre, the nuclei eventually move, in large numbers, into the interior. This begins at about the 100-fibril stage, but is not

complete till after the full number of fibrils has formed. It has the effect of splitting the fibre into columns of fibrils separated by accumulations of sarcoplasm, within which the large nuclei lie (figure 191). Later the nuclei diminish much in size, so that in the adult fibre the sarcostyles are again fairly evenly distributed. Very rarely a muscle nucleus is seen in mitosis. Sometimes degenerating nuclei are encountered. Both events must affect the numerical equality of nuclei and sarcostyles that we should have expected on developmental grounds.

The sarcosomes appear very late; I have not been able to derive them from any visible cell component of the young fibre.

The fibrils throughout the nymphal period are delicate threads, that thicken into sarcostyles only shortly before the final moult (compare, for example, figures 191 and 181). Striations increase terminally in number (about seven-fold), as the thorax enlarges to its adult size. A noteworthy point is the presence of shortened (contracted) fibres in the fixed preparation; in the adult fibre such zones of shortening are, of course, never found. Development of the tonofibrillae is essentially as in *Chortoicetes*.

In the earlier nymphal instars branches from the main mesothoracic trachea grow into the developing wing muscle, but penetration into the fibres begins only in the final instar. Silver impregnation shows swarms of branching tracheal cells that migrate from the large vessels and spread on to the young fibres. Figure 184, for example, shows a section along the oblique muscle, with tracheal cells spreading away from the large transected vessel above the muscle; note how the cells retain their connexion with the parent vessel. From these tracheal end-cells the intracellular vessels arise, either alone, or by penetration of the entire cell into the muscle fibre (figure 185). In either case the branching is confined to the zones of intercolumnar sarcoplasm, which accounts for the tendency of the tracheae of the adult fibre to expand in planes among the sarcostyles. Figure 185 shows the beginning of the branching. As it progresses a surprisingly dense tracheal network develops. But at the end of the nymphal period this becomes much reduced by the general expansion of the fibre.

B. *Wing-adjustor muscles*

I select for convenience the pleuro-tergal muscle of *Erythroneura*, to provide comparison with the wing vibrators. The parent myoblasts are distinguishable in the young nymph, as a clump of cells alongside the thick epidermis from which the wing buds will soon form. In the half-grown nymph this has developed into a thick clump of granular spindly cells that span the wing base (figure 173, plate 26). Within the myoblasts the granules now coalesce to form fibrillar substance. A variable number of such myoblasts then become associated into a single unit, with fibrillar substance merged into an axial column; in figure 174, for example, the group of myoblasts to the left (asterisk) could be interpreted as a phase in the fusion, which is complete in the young fibre to the right (two asterisks). Only then do the cross-striations appear. In the last instar the nuclei multiply, and the fibril column becomes much enlarged.

The above description is very similar to that given by Schaxel (1912) for myogenesis in the embryo of the annelid *Aricia*.

Other Jassidae

(1) *Myology*

For comparison with *Erythroneura* I have dissected the following: *Idiocerus* sp., *Tettegonia parthaon*, *Stenocotis costalis*, *Eurymela distincta*, *Anipo brunneus*, *Thymbris* sp., *Ulopa* sp., *Austroagalloides* sp., *Tartessus* sp., *Euscelius* sp., *Aetalion reticulatum*.

In this representative lot, the character of the musculature is essentially as in *Erythroneura*, though often much more strongly developed; in *Anipo brunneus*, for instance, the dorsal longitudinal muscle is carried by the downgrowing phragma almost on to the floor of the segment (figure 170, plate 26). Points of difference have appeared in the basalar muscle: in *Idiocerus*, *Austroagalloides* and *Tartessus* it is attached below, not to the basisternum, but to the rim of the coxa; in *Stenocotis*, *Thymbris* and *Aetalion* there are two basalars, one ending on the sternum, the other on the rim of the coxa.

(2) *Histology*

Here the principal difference relates to the fibre components of the muscles. *Erythroneura* has proved to be unusually simple, in that entire muscles consist usually of a single fibre. In the bigger jassids this is not so: for example, in *Anipo brunneus* the longitudinal muscle consists of some sixty fibres, in *Tettigonia*, *Austroagalloides* and *Stenocotis* of fifty to seventy, while in the large *Eurymela distincta* there are over 200; but in all these the fibres are grouped into five bundles. In others, again, there is a single tier of fibres ungrouped, seven in *Eurinoscopus*, fourteen in *Idiocerus*, while in *Euscelius* there are over seventy very delicate fibres.

In all the above the fibres fall into the category of coarsely fibrillar, with, however, a very wide range of sarcostyle thickness. In *Eurinoscopus* they are not more than $1{\cdot}5\mu$ thick; in some species of *Eurymela* exceptional sarcostyles may be as much as 5μ thick. In some species the sarcostyles are grouped into columns, recalling the immature fibres of *Erythroneura*.

(3) *Development*

A brief examination of various available jassids has shown that the type of myogenesis displayed by *Erythroneura* is widespread in the group, and probably general. This statement is based on the following material: *Idiocerus* sp., *Eurinoscopus viridis*, *Nesoclutha obscurata*, *Anipo brunneus*, *Stenocotis costalis*, *Tartessus* sp., *Eurymela albocincta*. Actually quite a small range of nymphs suffices to bring out at least the salient features of the myogenesis—viz. the gradual building up of the fibre with initially few cross-striated fibrils, by a process of progressive myoblast incorporation; and on these points all the above conform.

I have made a more detailed study of two species, of which I have had an abundant supply of nymphs.

(*a*) *Eurinoscopus viridis*

The myoblasts of the longitudinal muscle lie alongside certain tergal muscles in the young nymph. After a short initial period of proliferation, the pioneer myoblasts appear, extending the full length of the segment, as in *Erythroneura*. The early phases of fibril increase ensue, but my preparations do not show cross-striations until the five-fibril stage, when faint cross-membranes are also perceptible.

Figure 194, plate 27, represents a typical longitudinal section from a half-grown nymph. Three developing fibres are shown, and though a sarcolemma is not visible, the reader should have little difficulty in distinguishing myoblasts from muscle nuclei, the former being mostly short bipolar spindly cells, with an aggregation of dark cytoplasm at the poles. Above, the section grazes the myoblast investment of yet another fibre, but the fibre itself is not in focus.

There is the usual difficulty in actually observing the process of myoblast incorporation. Direct association of spindly myoblasts with the surface of the fibre is often seen, and in places we even get the impression that delicate filaments are growing from the myoblasts along the fibre. With phase contrast they are more effectively shown; for example, in figure 195 the myoblast marked with an asterisk has begun to elongate at each end, and even without change of focus can be traced along the fibre for about a fifth of its length.

A nucleus:sarcostyle count, made on three fibres chosen at random, gives the following result: 1730:1564, 3932:3833, 2715:2595. The numerical equality is so close as to indicate that each myoblast, as in *Erythroneura*, contributes a single sarcostyle to the fibre.

(*b*) *Anipo brunneus*

This is a medium-sized member of the eurymelid group of jassids, with multifibre structure of muscles; e.g. in the longitudinal muscle there are over sixty fibres, distributed in five bundles (figure 170, plate 26).

The development differs in important respects from that of *Erythroneura*, for the muscles arise out of embryonic muscle fibres, with which, however, free myoblasts soon become associated. In the smallest (1 mm) nymphs that I have obtained, the longitudinal muscle is represented by five, at times apparently only four, such fibres. They have a width of 4 to 5μ, and lie just median to the main trachea. They are enclosed in sarcolemma; nuclei are usually abundant; the fibrils lack cross-striations. The free myoblasts, at first very scarce, are scattered mainly along the under-side of the muscle rudiment.

Soon the diminutive fibres enlarge, and cleavage of their increasing fibril content sets in; an early phase is shown in figure 196, plate 27, a later stage in figure 197. By this means, and in the still quite small nymph, the rudiments of the future muscle are produced; in figure 196, for example, they number about twenty-five; in figure 197 about forty. The myoblasts, now more numerous, have meantime begun to invade the groups of fibril bundles (figure 198), and the process of myoblast incorporation then begins.

The rudimentary fibres now show weak cross-striation, but it remains faint till late in development, and is therefore not constantly seen (e.g. figure 199). Some of the myoblasts now become spindly (figure 198), in preparation for their incorporation into the growing fibre. *Anipo* has proved unusually helpful in elucidating the process, for in some preparations the fibril outgrowths can be traced for very considerable lengths along the young fibres. They are, however, very delicate, and in photographs (but not in the actual preparation) may be taken for clefts among the young fibres. An example is shown in figure 199; the parent myoblast is indicated by an asterisk, and its prolongation can here be followed for what is actually about a quarter the length of the entire muscle rudiment.

The later phases present little of note. Nucleus:sarcostyle counts, made on four adult fibres, have given the following results: 992:798, 1089:782, 988:708; 635:463. The

nuclei are here considerably in excess of sarcostyles, and this suggests that some of the myoblasts merge in chains rather than singly (the initial embryonic fibres do not seem to contribute many nuclei).

RICANIIDAE

Scolypopa australis Walker

This is the familiar 'passion vine' leafhopper of Australia. All stages from minute nymph to adult can be obtained, at times, on 'Virginia creeper' (*Ampelopsis hederacea*). The imago measures about 6 mm. Usually it sits at rest on foliage, and if disturbed hops with its powerfully developed springing legs. Flight is only feeble. I have not been able to determine the frequency of wing beat, because the insect will not fly in captivity.

(1) *Myology* (figure 12)

The flying muscles are weakly developed, especially in the metathorax.

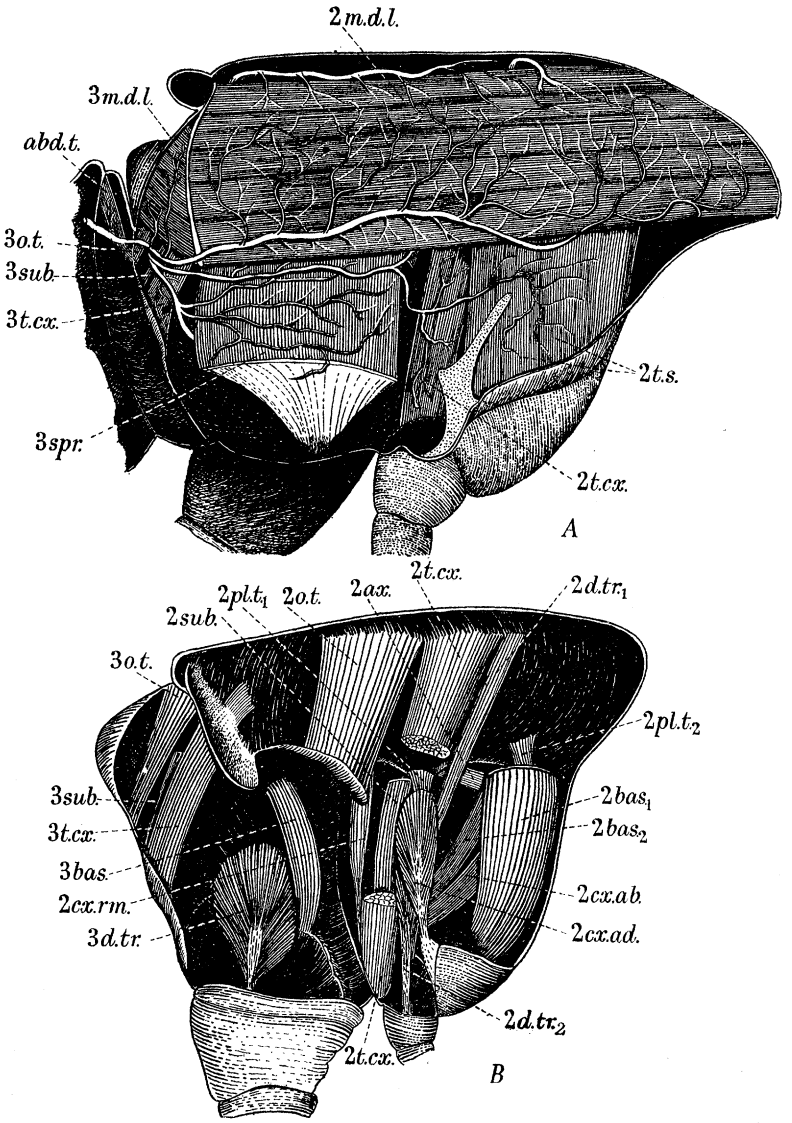


FIGURE 12. *Scolypopa australis*. *A*, median bisection of pterothorax; *B*, dissection, showing lateral muscles.

Mesothoracic muscles

A. *Tergal muscles*

(i) *Median dorsal longitudinal muscle* (2 *m.d.l*), a moderately developed muscle, composed of five subsidiary muscles; main wing depressor.

(ii) '*Oblique*' *tergal muscle* (2 *o.t*), arising from the phragma; upper attachment to hinder part of scutum. As in other Homoptera it has, by change of position, become an antagonist of (i).

A very short oblique muscle (shown in figure 12*B*, but unlabelled) is not a flying muscle.

B. *Dorso-ventral muscles*

(iii) *Tergo-sternal muscle* (2 *t.s*), a well-developed muscle, upper attachment over a considerable area of prescutum above wing base; lower to bulging basisternum. It is the main wing levator, and is actually in two parts, the hinder being perhaps the equivalent of the tergo-coxal of jassids, which has acquired a sternal attachment.

(iv) *Tergo-coxal muscle* (2 *t.cx*), moderately developed, attached below to meron of coxa. Since it does not mature till late in nymphal period, and since it has the histology of wing muscle, it is probably exclusively used in flight (wing levator).

There are two other dorso-ventral muscles, viz. a tergo-trochanteral muscle (depressor of trochanter, 2 $d.tr_1$), and a second tergo-coxal (coxal remotor, 2 *cx.rm*); both are functional leg muscles in the nymph, and as they lack the special histology of flight muscles, are probably pure leg muscles. It will be recalled that in cicadas the tergo-trochanteral is a flight muscle. Note that the first tergo-coxal of preceding species is absent, even as a functional leg muscle.

C. *Pleural muscles*

(v) *First basalar muscle* (2 bas_1), a broad muscle passing down from the basalar sclerite to its attachment on basisternum lateral to (iii); 'direct' wing depressor.

(vi) *Second basalar muscle* (2 bas_2), a much thinner muscle, with lower attachment to rim of coxa; 'direct' wing depressor.

Note absence of a third basalar, operating on trochanter.

(vii) *Subalar muscle* (2 *sub*), lower attachment to meron of coxa, upper to subalar sclerite; 'direct' wing depressor.

D. *Wing adjustor muscles*

(viii) *First pleuro-tergal muscle* (2 $pl.t_1$), arising from pleural ridge: tilts wing up.

(ix) *Second pleuro-tergal muscle* (2 $pl.t_2$), a short stout muscle arising from basalar sclerite, with similar function (not found in Orthoptera, but there is a minute similar muscle in cicadas—see figure 7*B*, unlabelled).

(x) *Axillary muscles* (2 *ax*), two muscles arising high up on the episternum, and attached to third axillary sclerite.

Metathoracic muscles

A. *Tergal muscles*

(i) *Median dorsal longitudinal muscle* (3 *m.d.l*), a short stout muscle, very obliquely placed under roof of segment, and composed of three subsidiary muscles; wing depressor.

(ii) *Oblique tergal* (3 *o.t*), weak wing depressor.

B. *Dorso-ventral muscle*

(iii) *Tergo-coxal muscle* (3 *t.cx*), a rather weak muscle, placed obliquely across segment; lower attachment to upper end of (fixed) coxa; wing levator.

The huge springing muscle (3 *spr*) operates by a powerful tendon on the trochanter, and is the tergo-trochanteral of this segment.

C. *Pleural muscles*

(iv) *Basalar muscle* (3 *bas*), a single thin muscle attached below to anterior rim of coxa; 'direct' wing depressor.

(v) *Subalar muscle* (3 *sub*), lateral to (iii); weak 'direct' wing depressor.

D. *Wing adjustor muscle*

(vi) *Axillary muscle*, very small, and attached to third axillary sclerite.

Abdominal wing muscle

A tergal muscle in the abdomen (*abd.t*), appears to be an accessory flight muscle. It is an intersegmental muscle, probably the dorsal longitudinal drawn out of position, with anterior attachment to the metathorax where the oblique muscle arises. It develops in the nymph, its fibres have intracellular tracheae, and in its histology it approaches the other flight muscles though the fibres are smaller. Since it pulls in the opposite direction to the metathoracic oblique muscle, it could be an accessory wing levator.

In Delphacidae (described below) the accessory abdominal flight muscle is much better developed.

There is, of course, some uncertainty about the functioning of potential flight muscles when they are associated with the basal joints of the leg. The reason for regarding the above muscles as flight muscles, and, indeed, as exclusive flight muscles, is their late appearance and their histology. As with the cicada, those associated with the leg are plainly homologous with certain muscles of Orthoptera, where, however, they function in the double role of leg and flight muscle. But in *Scolypopa* the tendency to assume exclusively the new role of flight muscle is less than in cicadas, perhaps because of the freer life that the nymph leads. Thus the tergo-trochanteral is an exclusive leg muscle, being in the metathorax the huge springing muscle. The apparently exclusive flight muscles associated with the leg base are: in mesothorax, numbers iv, vi, vii; in metathorax, numbers iii, iv, v. Deprived of their use the nymph, and probably adult, utilize certain other exclusive leg muscles; conspicuous in the mesothorax are the coxal abductor (2 *cx.ab*), coxal adductor (2 *cx.ad*) and depressor of trochanter (2 $d.tr_2$), and a large tergo-coxal remotor (2 *cx.rm*). In the metathorax, where the coxa is very large but fixed, the leg muscles act only on the trochanter. The largest, apart from the springing muscle, is a depressor of the trochanter (3 *d.tr*).

Tracheal supply

The main trachea of the thorax is unusually narrow, and lies lateral to the median dorsal longitudinal muscle. In median bisections of the thorax only subsidiary branches are seen. Of these the largest is a vessel that runs along the lower surface of the longitudinal muscle, to which it supplies numerous branches, and then passes into the pro-

thorax. A smaller vessel goes to the tergo-sternal and tergo-coxal muscles, and yet another to the large springing muscle. The general character of the branching is shown in figure 12*A*. The main trachea supplies the lateral wall of the median dorsal longitudinal muscle with numerous fine branches, and it is from this vessel also that the more laterally placed wing muscles are supplied.

(2) *Histology*

Cross-sections of entire muscles show a peculiar fibre disposition, the fibres tending to lie in a ring; and although there are departures from the pattern, yet it is plain from any cross-section (figure 200, plate 27) that the fibre grouping is a most unusual one.

The fibres, as figure 200 shows, vary from rectangular to polygonal in cross-section, and range between 0·02 and 0·04 mm in maximum width. The sarcolemma is best seen in frozen sections of fresh tissue, and in fixed material is often invisible (e.g. figure 201). The nuclei usually lie just below the sarcolemma.

The sarcostyle pattern is shown in figure 201. Even more than in cicada muscle do the sarcostyles tend to lie in rings in the cross-section. But the elegant lamellar pattern of cicada muscle is here replaced by a more massive grouping into thick columns, irregular both in form and in size. In fixed preparations the intercolumnar spaces are considerable, but as comparison with frozen sections shows, are much enlarged by fixation.

The fresh relaxed sarcostyle measures under 1 μ thick. Figure 203 (plate 28), shows the appearance of the relaxed fibre in a preparation in which the *Q*-bands are evident. In cross-section most of the sarcostyles appear triangular (figure 201), and in exceptionally good preparations are, as expected, resolved into three myofibrils. The sarcostyles are, in fact, similar to those of the closely related *Siphanta acuta*, which have already been described in detail above. The inter-*Z* distance is about 4 μ in the relaxed condition, being reduced to about half at maximum contraction.

From the character of the myogenesis, described below, we should not expect a numerical equality between the nuclei and sarcostyles; the following counts serve therefore to emphasize the difference in this respect between the *Scolypopa* and jassids:

muscle	fibre length (mm)	no. of nuclei	no. of sarcostyles
dorsal longitudinal (mesothorax)	1·75	762	1620
	1·75	483	1180
dorsal longitudinal (metathorax)	0·45	306	1835
	0·37	252	1920
oblique tergal (metathorax)	0·8	354	2820
tergo-coxal (mesothorax)	0·73	351	1710

The sarcosomes are transversely alined, there being a double row to each cross-band.

Of the cross-membranes, a complete *Z*-membrane, transecting not only the interfibrillar spaces, but even the intercolumnar spaces, has at times appeared in my preparations. At the margin of the fibre it produces the familiar 'festooning' of the sarcolemma, best seen in contracted fibres. *Mf* is more elusive, and I have only occasionally had a hint of *Ms*.

Regarding the pattern of the cross-striation, there is really no difficulty, in focusing through the thick fibres, of seeing the usual helicoidal appearances. But the fibres are

easily distorted, and the complete pattern of the whole fibre cannot be determined. The impression is given, in focusing through the fibre, of more than one helicoidal system at different levels of focus.

The tracheal supply is not rich, for the insect is a very poor flier. As in cicadas the tracheae are confined to the narrow clefts between the columns of sarcostyles, sections along the clefts therefore showing the full expanse of the tracheal net, almost without focal change; in figure 202, plate 27, two adjacent fibres have accidentally been cut in the appropriate plane. Sometimes the tracheal end-cells lie actually below the sarcolemma, sometimes outside it. There are no blindly ending tracheae, and even with unchanged focus, continuity between adjacent tracheae is often seen (e.g. figure 202, the tracheae entering at point marked by asterisk).

With the Da Fano process, the innervation of the muscle is also often shown. The branching nerves that arise from the thicker nerve trunks usually contain two nerve fibres, and though one is thicker than the other, the disparity is much less than in the cicada (above described). Both fibres branch simultaneously, and in a few instances I have been able to see the actual motor endings. In the formation of the end-organ both fibres participate, the nerve terminals being exceedingly delicate twigs, almost unbranched, lying in a just recognizable aggregation of sarcoplasm below the sarcolemma.

(3) *Development*

The following description is given with special reference to the dorsal longitudinal muscle of the mesothorax, but applies in general to all the flight muscles, including the abdominal.

The wing muscles develop, as in jassids, out of free myoblasts, and not from pre-existing nymphal muscles, as in most of the foregoing types. In the smallest nymphs that I have obtained, measuring only 1 mm long, development is already under way. The myoblasts number about twenty, and lie just under the roof of the segment alongside the main trachea, and of these a few have already grown long and filamentous, to become the pioneer myoblasts for the future muscle. These differ, however, in one important respect from those of *Erythroneura*, in that several co-operate in a chain to form the initial rudiment of the fibre. In figure 204, plate 28, representing about half the length of such a fibre rudiment, two myoblasts are visible; in those that arise later they are certainly more abundant (figures 205, 206).

My preparations show with unusual clarity, that within the pioneer myoblast, the initial fibril arises by coalescence of cytoplasmic granules that spread along the delicate prolongations of the myoblasts. Figure 204 shows the granules; the completed fibre rudiment with single axial fibril is shown in figures 205 and 206, and as figure 206 shows (marked by asterisk) the fibril already has faint cross striations.

In the rather later nymph from which figures 205 and 206 are taken, the myoblasts have increased to about a hundred, five (possibly six) such rudimentary fibres being already present. As figure 206 shows, the myoblasts lie mostly below the developing fibres, but are not always readily distinguishable from muscle nuclei; in figure 206 several are at once evident by their spindly form, and to the extreme right of the figure one is undergoing elongation.

The myoblasts continue to increase in number, but I have had some difficulty in following the exact course of events at this period of development. Cross-sections from only slightly more advanced nymphs show the muscle rudiment as a thin but compact band, attached to the medial wall of the main mesothoracic trachea (figure 207). At least eleven muscle fibres, distinguishable by their sharply staining fibrils, are now present. Whether there has already been some fibre cleavage is uncertain; there can, however, be no doubt that it does now take place, and that by repeated division of the initial fibres, the rudiments (numbering about 90) of the fibres that comprise the adult muscle will eventually appear.

If figure 208, from a slightly larger nymph, is compared with figure 207, an increase of fibre number in the former, up to about fifty, is apparent, and this increases to the full number of about ninety in figure 209. Comparison of the three figures shows that fibre increase is attended by cleavage of the original muscle rudiment into five columns, each the forerunner of the five above-described muscles; and that in the processs the daughter fibres arrange themselves around an axial column of myoblasts, and so initiate the peculiar fibre pattern that distinguishes the adult muscle (figure 200). Already in figure 207 there seems to be an indication of cleavage into three columns, and the impression is given that in the column nearest the trachea some muscle fibres are already moving down to enclose the myoblasts from below.

Throughout the whole of this period the fibrils are sharply cross-striated. From the very beginning also, there is evidence of fibril proliferation within the rudimentary muscle fibres. But before the fibril bundle attains any considerable magnitude, it is reduced by cleavage. For example, in figure 208 (with unresolved fibrils) the bundle indicated by a single asterisk has considerably enlarged; the double asterisk points to a case of probable cleavage. With the completion of fibre cleavage, the rudiments of the definitive fibres are left often, perhaps always, with but a single fibril, which, however, soon enters into cleavage again (figure 210).

This completes the first phase of the myogenesis, the nymph being less than half-grown. We enter now upon the second phase, in which the free myoblasts become incorporated into the fibre rudiments. The reader who attentively examines the cross-sections that accompany this description (figures 209, 211 to 213) will have some difficulty in seeing any distinction between free myoblasts and nuclei of the rudimentary muscle fibres. It should be explained that the myoblasts are mostly short bipolar cells, lengthwise orientated (figures 214, 218), and are therefore not easily recognized in cross-sections. In figure 209 the nuclei around the margin of the fibre columns are plainly muscle nuclei; the nuclei in the interior of the columns are nuclei of free myoblasts, and several of these, even in cross-section, are recognizable as free cells by their investment of cytoplasm.

In the half-grown nymph proliferation of the myoblasts becomes greatly accelerated, and an astonishing wealth of mitoses is sometimes encountered (figure 219, plate 29); but any heaping up of myoblasts is soon offset by their concurrent incorporation into the growing fibre.

The rudiments of the definitive fibres, as we have seen, contain only a few fibrils, and commonly only one (figures 209, 210). They are from the beginning cross-striated, and even minute *Zf*-disks are visible (figures 217 to 218*a*). The early fibril increase that is

indicated in figure 209 probably takes place wholly by fibril cleavage, the cleavage being directly observable in the longitudinally cut tissue. From the beginning the cross-bands of adjacent fibrils are alined, and in favourable places a complete *Zs*-membrane can be seen even in the two-fibril stage (figure 218*a*). Each rudimentary fibre has, moreover, its sarcolemmal sheath; but as figure 222, plate 29, shows, the sheath leaves the substance of the fibre exposed where it abuts on the axial column of myoblasts.

It is along this exposed surface that the myoblast intake must proceed; but there is the usual difficulty of directly observing the process. The general character of the developing muscle, at this period, is shown by figure 214, the photograph showing the multitude of spindly myoblasts that now swarm along the developing fibres. Where crowding of myoblasts is not excessive, we see individual cells prolonging lengthwise into long delicate tendrils (figure 215, marked by asterisk), and in favourable places end-to-end fusion of myoblasts is seen (figure 216). Along these tenuous filaments the cytoplasmic granules, hitherto confined to the vicinity of the nucleus (figures 218, 221), spread, and then presumably fuse into a single continuous fibril. It is just this final stage, in which the myoblast becomes part of the fibre, that is most difficult to recognize in fixed preparations. Figure 220 (drawn in figure 220*a* with the advantage of focus adjustment) shows a single fibril, deeply staining and without cross-striation, still part of a myoblast, but so closely merged into the young muscle fibre as to be a part of it. This we interpret as the last recognizable stage in the incorporation of the myoblast, the completion of which must necessarily obliterate all evidence of its extraneous origin.

The course of events suggested by these disconnected pictures is the following: the free myoblasts, prolonged into delicate filaments along the inner margin of the fibre, merge with the latter, not singly along its whole length, but as a succession of myoblasts, which bring with them a number of nuclei, some sarcoplasm and a single fibril, the latter acquiring a cross-striation that adapts itself to the pattern already existing in the growing fibre.

Fibril cleavage continues during the period of myoblast intake, and it is presumably to this that a well-ordered fibril pattern, that now appears, is due. In the earlier cross-section shown in figure 209, the only grouping of fibrils is an occasional tendency to lie in half-rings. In the later phase shown in figure 211 the rings have closed, there is evidence of fibril splitting, and here and there a product of the cleavage has moved into the middle of the ring. A pattern of grouped fibrils is thus built up (figure 212), and as this becomes further elaborated (figure 213) we see the first signs of the columns of the adult fibre. Fibril grouping in rings is retained even in the adult fibre (figure 201), and is presumably a consequence of fibril cleavage.

Z-membranes, transecting the interfibrillar spaces, are present from the beginning (figure 218*a*), but with present methods cannot be consistently shown. A helicoidal pattern is present already at the thirty-fibril stage (figure 221) or earlier, i.e. long before the completion of myoblast intake (note free myoblasts in figure 221).

The sarcosomes, as usual, appear only late in development.

The sarcolemma, the early appearance of which has already been noted, is plainly a plasma membrane. Complete enclosure of the fibre is necessarily delayed till after myoblast intake is completed.

Development of the tracheae can be followed in Da Fano preparations. During the earlier phases of development, tracheae grow in increasing numbers into the tissue, but their actual penetration into the fibres is deferred to the final phase of muscle-fibre enlargement. The general picture is similar to that of *Erythroneura*, above described.

FLATIDAE

Siphanta acuta Walker

This insect, with all larval stages, can be obtained locally from the fresh shoots of many garden shrubs. It is about the same size as *Scolypopa*. It flies more readily when disturbed. The frequency of wing beat is 37 to 41/s. The wing muscles, including accessory abdominal muscle, are as in *Scolypopa*. Their histology and development also are similar. The only observed difference is that in *Siphanta* the muscles develop later than in *Scolypopa*.

I have had available for dissection a number of other flatids: *Paratella fumaria*, *Hansenia glauca*, *Paradaksha meeki*, *Euphanta ruficeps*. The general plan of the musculature is in all very similar to that of *Siphanta*.

DELPHACIDAE

Perkinsiella saccharicida Kirkaldy

This is the notorious sugar-cane leafhopper of Queensland. My material has been kindly sent me by the Bureau of Sugar Experimental Stations, Brisbane. The insect measures 3 to 4 mm long (excluding wings). The male is fully winged; some of the females, also, are winged, but others have tegmina only, and do not fly.

(1) *Myology* (figure 13)

Mesothoracic muscles

A. *Tergal muscles*

(i) *Median dorsal longitudinal muscle* (2 *m.d.l*), attached behind to the phragma, and only moderately developed; wing depressor.

(ii) '*Oblique*' *tergal muscle* (2 *o.t*), not strongly developed; attached below to the forwardly curving phragma, and passing almost vertically up to its attachment on scutum; wing levator.

B. *Dorso-ventral muscles*

(iii) *Tergo-sternal muscle* (2 *t.s*), a moderately developed muscle, with lower attachment to basisternum. As in other Homoptera already considered, this muscle is in two parts, the hinder being possibly an originally tergo-coxal muscle.

This is the only dorso-ventral flying muscle; there is no muscle equivalent to the tergo-coxal flying muscle of *Scolypopa*. The other dorso-ventral muscles are purely leg muscles, viz. a tergal promotor of coxa (2 *cx.pr*) not present in *Scolypopa*, a tergal depressor of trochanter (2 *d.tr*), and a tergal remotor of the coxa (not visible in figure).

C. *Pleural muscles*

(iv) *Basalar muscle*, a single weakly developed muscle, with lower attachment along lower margin of episternum; 'direct' wing depressor (not visible in figure).

There is the usual coxal abductor (pleural) muscle, to the side of the basalar.

(v) *Subalar muscle*, small and attached to rim of coxa; 'direct' wing depressor (not visible in figure).

D. *Wing adjustor muscles*

These are similar to those of *Scolypopa*.

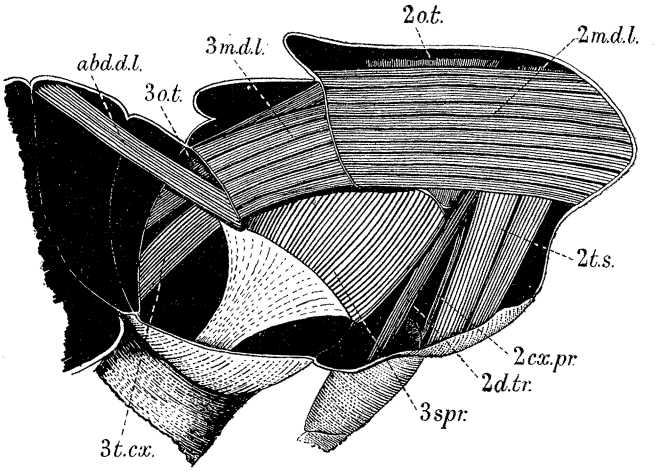


FIGURE 13. *Perkinsiella saccharicida.*

Metathoracic muscles

A. *Tergal muscles*

(i) *Median dorsal longitudinal muscle* (3 *m.d.l*), a short stout muscle, attached at either end to the phragma; wing depressor.

(ii) *Oblique tergal muscle* (3 *o.t*), a very laterally placed muscle; wing depressor.

B. *Dorso-ventral muscles*

(iii) *Tergo-coxal muscle* (3 *t.cx*), a rather weak muscle running obliquely across the segment, and attached below to posterior rim of coxa.

Note, as in *Scolypopa*, the absence of a tergo-sternal muscle. There are, of course, no tergal muscles operating on the coxa, for this is fixed. The principal dorso-ventral muscle is the large obliquely placed springing muscle (3 *spr*), operating by a strong tendon on the trochanter.

C. *Pleural muscles*

(iv) *Subalar muscle*, lying in an oblique position lateral to (iii) (not visible in figure).

There are no basalars.

D. *Abdominal muscles*

In *Perkinsiella*, as in *Scolypopa*, a tergal abdominal muscle (*abd.d.l*) is co-opted as a flying muscle, but in a more remarkable manner. It is a rather narrow but long muscle that arises from the hinder margin, not of the first but of the second abdominal segment, and has its anterior attachment to the phragma. It is presumably an indirect wing levator, for its contraction must exert on the metathorax the opposite effect to that of its own dorsal longitudinal; indeed, the only other wing depressor in the metathorax is the weak subalar.

The abdominal flying muscle is present in the male, and in the winged but not unwinged female. Histologically it falls, like the other flying muscles of this insect, into the category of coarse fibrillar (Siebold) muscle.

(2) *Histology*

The fibres are mostly polygonal or rectangular in cross-section, ranging from 0·07 to 0·1 mm in width, and fit closely to form a compact muscle, without the fibre pattern found in *Scolypopa* and *Siphanta*; in the mesothoracic dorsal longitudinal, for instance, there are about two dozen fibres, firmly wedged together in a narrow band, averaging two fibres in width. All the flight muscles are similarly constructed except the accessory abdominal; the latter is a rather thin bundle of some thirty unusually narrow fibres, only about 0·016 mm in width.

The sarcostyles, fairly evenly distributed in the cross-section, are of the coarse (Siebold) type, measuring in most fibres about 2 to 2·5μ thick. But in the smaller metathoracic and accessory abdominal muscles they are a little thinner. The inter-Z distance is 2μ. Of the nuclei, about half lie just below the sarcolemma; the others are distributed mostly in rows between the sarcostyles, commonly lying in very narrow clefts, occupied by sarcoplasm that intrudes between the sarcostyles.

A nucleus:sarcostyle count in a fibre of the mesothoracic dorsal longitudinal muscle gave a value 1040:935; for a fibre from the corresponding metathoracic muscle, which is about half the length of the former, it gave 835:1605.

The sarcosomes are transversely alined in a double row to each striation.

The tracheae are weakly developed, as in other Homoptera already described.

(3) *Development*

The wing musculature arises out of free myoblasts. Those of the mesothoracic longitudinal muscle are the most easily recognized, for they lie midway along the main trachea, just under the roof of the segment. In the youngest nymphs that I have examined there are only eight such cells, and of these five have begun to elongate into pioneer myoblasts. Figure 223, plate 29, shows a section along the roof of the mesothorax of this nymph; it has been photographed at two slightly different foci, in order to maintain the focus of one of the pioneer myoblasts. The latter has become prolonged into a most delicate filament, but so far at its hind end only, where it reaches the hind limit of the segment. The cells below it are tracheal cells, the section grazing the main trachea.

The pioneer myoblasts in this species all seem to span the length of the segment singly, as in jassids, and not in chains, as in *Scolypopa*. Cytoplasmic granules spread along the filaments, and by coalescence form the first fibril.

The free myoblasts now rapidly increase in number, and accumulate into a clump midway along the under-surface of the muscle rudiment, but from where they soon spread almost evenly along its length. A thin band of developing muscle thus arises, traversing the length of the segment along the medial wall of the main trachea.

I have had much difficulty in following the exact course of events at this early period of development. Little can be made out in the cross-section except that it is traversed by five enlarging bundles of most delicate fibrils; but whether these have arisen wholly by cleavage of the original five fibrils or, in addition, by myoblast incorporation is not clear. In

nymphs that are only slightly more advanced, the muscle rudiment has split into five separate columns (figure 224, plate 29); each is enclosed in a limiting membrane (sarcolemma), and is plainly a rudimentary multinucleate muscle fibre, though without cross-striations. Adhering to the young fibres are scattered myoblasts.

Here, and in the immediately succeeding stages, we are reminded of the early development of *Anipo*, described above. Within the rudimentary fibres the fibrillar substance splits into sub-columns (indistinctly seen in figure 224,) and between these daughter fibres the free myoblasts insinuate themselves. The five columns thus come to consist of a peripheral zone of growing fibres, enclosing an axial core of free, but soon very crowded myoblasts, and within the young fibres cross-striations are now distinct (figure 225). This early cleavage into the usual five developing fibre bundles was unexpected, for there is certainly no such fibre grouping in the adult muscle. As figure 226 shows, it is actually retained for some time.

The usual myoblast incorporation now ensues, and this takes place much as in *Scolypopa*. The outer wall of the daughter fibres is invested in sarcolemma (figure 226), but the inner surface, where it abuts on the axial column of myoblasts, is exposed, and here the myoblasts are added to the growing fibres. Cross-sections show fibril grouping, as in *Scolypopa*, indicative of fibril cleavage. When we couple this with the nucleus:sarcostyle ratio given above, we have to conclude that the myoblasts are built into the growing fibre in chains, as in *Scolypopa*, and not singly as in *Erythroneura*. The later phases of the myogenesis do not call for comment.

The above description applies to all the flying muscles, including the abdominal. It is of interest to find that the early rudiments of wing-muscle fibres are found even in the wingless female, and persist in the adult.

CERCOPIDAE

Bathylus albicinctus Erichson

The nymphs of these strange and uncouth insects occur in abundance in early spring on their food plant *Plantago lanceolata*, the juice of which they suck while enveloped in a protecting exudate of frothy 'spittle'. They are sluggish creatures, and only after their last moult do they acquire the ability to spring, which is their one effective means of escape. The male, though fully winged, cannot fly, for it is devoid of wing muscles; in the female a wing musculature is present, but never strongly developed. Both sexes raise the tegmina when handled, but even the females do not seem to vibrate the wings. The female measures 5 mm, the male a little less.

(1) *Myology* (figure 14)

Development of the flight muscles in the female ranges from moderate to feeble.

Mesothoracic muscles

A. *Tergal muscles*

(i) *Median dorsal longitudinal muscle* (2 *m.d.l*), weakly developed (figure 230 plate 29), attached behind to phragma, its fibres being disposed into three or four bundles, below which is another narrow strip ('aberrant muscle') (2 *ab*), distinguishable by its white colour. The latter alone is present in male; function in either sex obscure.

(ii) *Oblique tergal muscle* (2 *o.t*), lower attachment to phragma, upper to lateral part of scutum; absent in male.

B. *Dorso-ventral muscles*

(iii) *Tergo-sternal muscle* (2 *t.s*), lower attachment to basisternum; absent in male.

(iv) *First tergo-coxal muscle* (2 $t.cx_1$), a slender muscle, attached below to rim of coxa; absent in male.

(v) *Second tergo-coxal muscle* (2 $t.cx_2$), a slender muscle, obliquely placed, attached below to meron of coxa; absent in male.

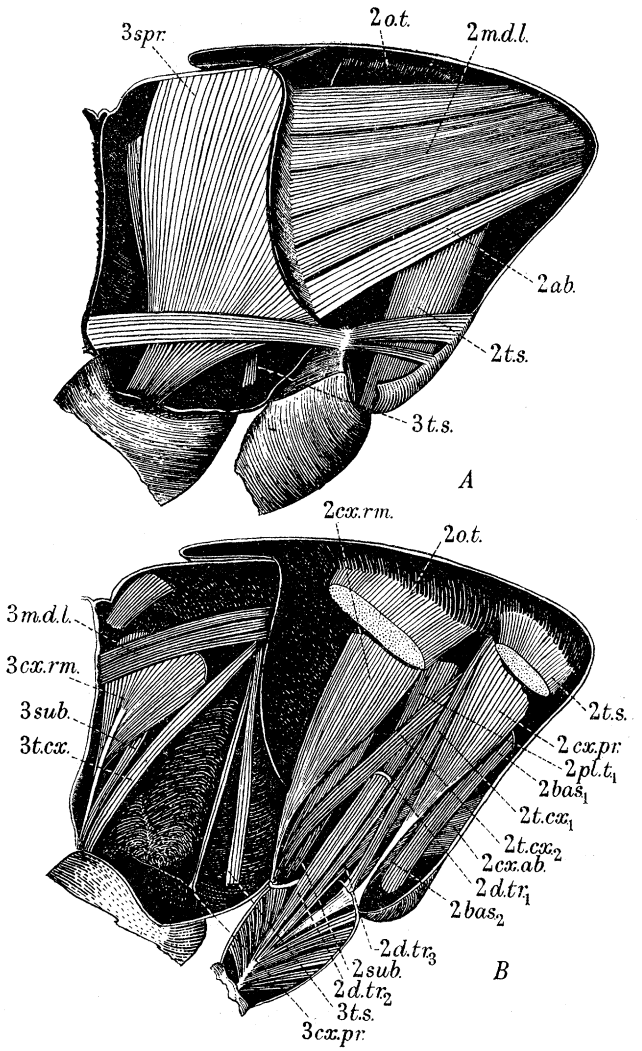


FIGURE 14. *Bathylus albicinctus*. *A*, median bisection of pterothorax; *B*, dissection to show lateral muscles.

The foregoing muscles, present only in female, have the yellow colour of flight muscles. Included in this group, and present in both sexes, are the following white muscles: (*a*) coxal promotor (2 *cx.pr*), a large muscle with long tendon, attached to coxal rim; (*b*) tergo-trochanteral (2 $d.tr_1$), depressor of trochanter, having a tendinous attachment to trochanter in common with a pleural muscle (2 $d.tr_2$); (*c*) coxal remotor (2 *cx.rm*), with tendon attached to hinder rim of coxa.

C. *Pleural muscles*

(vi) *First basalar muscle* (2 bas_1), attached below to basisternum, beside (iii).

(vii) *Second basalar muscle* (2 bas_2), attached below to rim of coxa.

The third basalar muscle (2 $d.tr_3$) is a white muscle, present in both sexes, and is not, therefore, a flight muscle. The usual coxal abductor (2 *cx.ab*) of this group is present.

(viii) *Subalar muscle* (2 *sub*), lateral to coxal remotor; lower attachment on meron of coxa.

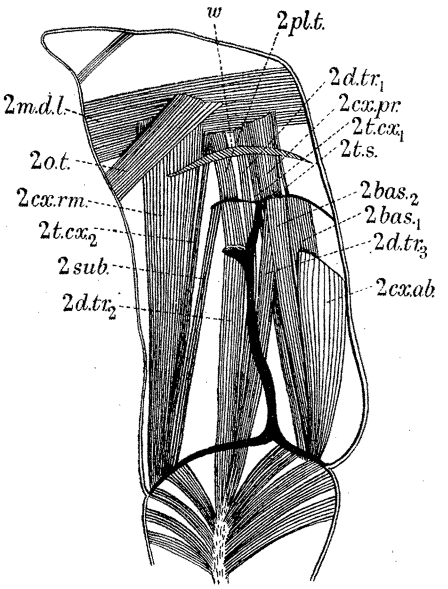


FIGURE 15. *Bathylus albicinctus*; young nymph. Muscles of mesothorax, seen from outside through transparent chitin. *w*, base of developing wing; other lettering in text.

D. *Wing adjustor muscle*

(ix) *First pleuro-tergal muscle* (2 $pl.t_1$), arising from upper end of pleural apodeme; present in both sexes; probably raises tegmen.

(x) *Second pleuro-tergal muscle*, arising from lower rim of episternum; probably raises tegmen.

(xi) *Axillary muscle*, attached to axillary sclerite.

Metathoracic muscles

A. *Tergal muscle*

(i) *Median dorsal longitudinal muscle* (3 *m.d.l*), very feebly developed.

B. *Dorso-ventral muscles*

(ii) *Tergo-sternal muscle* (3 *t.s*), very thin; attached below to basisternum.

(iii) *Tergo-coxal muscle* (3 *t.cx*), obliquely placed, attached to meron of coxa.

An exceedingly feeble coxal promotor (3 *cx.pr*) and a strong remotor (3 *cx.rm*) are included in this group; also the huge springing muscle (3 *spr*).

C. *Pleural muscle*

(iv) *Subalar muscle* (3 *sub*), attached to meron of coxa.

There are no basalars.

(2) *Histology*

This is not typical for cercopids; in various other species the muscles are of the coarse Siebold type.

In *Bathylus* the fibres of the dorsal longitudinal muscles range from 0·02 to 0·05 mm in diameter, but in exceptional individuals, with better developed muscles, may reach 0·1 mm. The nuclei are mostly very large, and usually lie just below the sarcolemma. The sarcostyles, except for an occasional tendency to group into columns around the fibre margin, are distributed without pattern in the fibre. They measure rather more than 1 μ thick, and in good preparations show two or three myofibrils. The inter-*Z* distance is as much as 4·5 μ (relaxed), reduced to half at contraction. Complete *Z*-membranes are often seen; *Ms* has not appeared in my preparations. The sarcosomes are very minute, longitudinally disposed without relation to the cross-striation. Tracheae, though abundant, do not penetrate into the fibres.

(3) *Development*

In *Bathylus* the flight muscles do not develop out of free myoblasts, but from pre-existing larval muscles, with which, however, free myoblasts later become associated.

Figure 15 shows, for comparison with figure 14, the mesothoracic muscles of a very young nymph, viewed, however, not in medial bisection, but through the transparent body wall. The relevant muscles are: A. TERGAL MUSCLES: (i) *median dorsal longitudinal* (2 *m.d.l*) and (ii) *oblique tergal* muscles, the forerunners of similarly named flight muscles of the adult; in the young nymph they must function as weak flexors of the thorax. B. DORSO-VENTRAL MUSCLES: (iii) *tergo-sternal* (2 *t.s*), presumably functionless in nymph, where it comprises but a single fibre lying along anterior margin of the coxal promotor; (iv) *coxal promotor* (2 *cx.pr*) and (v) *coxal remotor* (2*cx.rm*), both functional leg muscles and not the forerunners of any flight muscles; (vi) a thin tergal *depressor of trochanter* (2 $d.tr_1$), even in adult a pure leg muscle; (vii, viii) *first and second tergo-coxal muscles* (2 $t.cx_1$, 2 $t.cx_2$), two very delicate muscles, forerunners of similarly named flight muscles of adult; they comprise each only a single fibre, and lie alongside (iv) and (v), from which they are apparently derived; presumably they are functionless in the nymph. C. PLEURAL MUSCLES: the episternal group comprises: (ix) the usual *coxal abductor* (2 *cx.ab*), exclusive leg muscle; (x, xi) two smaller muscles (2 bas_1, 2 bas_2), of doubtful function in nymph, but subsequently enlarging to form the first and second basalar wing muscles; (xii) *depressor of trochanter* (2 $d.tr_3$), an exclusive leg muscle, equivalent of third basalar of many other types. Of the epimeral group there is only one, (xiii) the *subalar* (2 *sub*), thin and probably without function in the nymph, and subsequently enlarging into subalar wing muscle. In addition we find: (xiv) *depressor of trochanter* (2 $d.tr_2$), arising from pleural ridge, pure leg muscle, both in nymph and adult, and (xv) *pleuro-tergal* muscle (2 *pl.t*), from which the first pleuro-tergal wing adjustor arises.

The following account of the histogenesis is based on a detailed examination of the dorsal longitudinal muscle. The muscle comprises fifty to seventy fibres disposed in four bundles, below which are about seventy very thin fibres, constituting the 'aberrant' muscle above referred to. Below the latter is the main mesothoracic trachea (*tr*, figure 230, plate 29).

The six bundles of fibres arise out of the six nymphal fibres that constitute the dorsal longitudinal muscle of the mesothorax. A cross-section is shown in figure 227, the six fibres being indicated by the labelling lines from the asterisk. In their histology they present only one unusual feature; this is the presence of myoblasts beneath the sarcolemma. From the muscle nuclei the myoblasts are distinguished by their investment of cytoplasm. The latter is deeply staining and is aggregated at the ends of the cells, which are mostly lengthwise disposed. Note in figure 231 a muscle nucleus (*m.n*) to the left, and three myoblasts (*my*) to the right.

The conversion of these six nymphal fibres into the longitudinal wing muscle starts in the quite early nymph. It begins by enlargement of the individual fibres, beneath the sarcolemma of which a cleavage of their increasing fibril content then ensues; figure 234 (plate 30), for example, shows three fibres from the still quite small nymph, in this initial phase of the cleavage. A much later phase is shown in figure 233 (plate 29), representing a fragment of the cross-section shown at lower magnification in figure 229; figure 233 shows the progeny of the third and fourth muscle fibres, which have split into twelve and eight daughter fibres respectively, i.e. about two-thirds the definitive number of muscle fibres. A faint membrane still visibly encloses the bunch of developing fibres, and is plainly the original sarcolemma. A noteworthy feature is the presence of occasional nuclei within the membrane (one is indicated by asterisk); it seems that a few nuclei not distributed among the developing fibres remain with the investing sheath.

The reader will form a general picture of the early enlargement and general relations of the muscle rudiment from figures 227 to 229. In figure 228 derivation of the future muscle from the six nymphal fibres is plainly evident (they are indicated by asterisk; note trachea (*tr*) beneath them; note to left (crossed by labelling line) the developing oblique tergal muscle). In figure 229 the wing muscle proper has enlarged into a compact sheet of developing muscle (upper labelling line), separated by fat-body from the 'aberrant muscle' (lower line) adjacent to the trachea.

These events, occupying the early and middle nymphal periods, are attended by a pronounced weakening of the cross-striation, and therefore presumably of the internal differentiation of the fibrils; indeed, in many preparations there is no sign of any striation at all. But in exceptional preparations there is evidence that organization of the fibrils still persists, since in places we can detect the presence of faint *Q*-bands, and particularly of *Zs*-membranes, transecting the interfibrillar spaces (figure 237). Weakening of cross-striation attending abeyance of function has already been noted in some of the species examined above.

Activity among the myoblasts also begins early; figure 231 shows, for example, division of a myoblast even preceding cleavage into fibril bundles. A fragment from a rather later nymph is shown in figure 235; the distinction between muscle nuclei and myoblasts is here more evident, the myoblasts having a deeply staining though still rather sparse coat of cytoplasm around the nucleus. One myoblast is in mitosis. The photograph emphasizes the fact that the myoblasts lie internal to the feebly staining sarcolemma; it shows also that some of the myoblasts and muscle nuclei have now moved from the periphery of the fibre in between the daughter bundles. The distinction between myoblasts and muscle nuclei is plain also in figure 237.

The myoblast proliferation soon brings about a pronounced change in appearance of the tissue, and we are now struck by its richness in nuclear material (figure 238). Many of the nuclei are the nuclei of myoblasts; but even before the nymph is half-grown an increasing proportion have become purely muscle nuclei, till eventually the myoblasts completely disappear. The actual process of myoblast incorporation is as elusive as ever, and, indeed, for much the same reason as in the various other species considered above, for the dense crowding of myoblasts in the confined space between the fibril bundles usually precludes exact observation. In rather younger nymphs where the crowding is less, we commonly see the myoblasts elongating along the fibril bundles, and even uniting in chains (figure 236); or, again, we find a single myoblast prolonging into a most delicate filament along a fibril bundle (figure 232, asterisk). The cytoplasmic granules become spread along the filaments, and presumably then coalesce into a fibril which becomes built into the growing fibril bundle; but for reasons already discussed the final phase of myoblast incorporation is not amenable to observation.

The picture that emerges from the foregoing is as follows: beneath the sarcolemma of the enlarging nymphal fibres progressive cleavage of the fibril content takes place to produce eventually the definitive number of wing-muscle fibres. Though the cross-striation soon weakens, *Z*-membranes at least are retained, and so continue to impose an organization on the fibril content. Into this pattern new fibrils, brought by the myoblasts, are accommodated, and thereafter participate in the continued fibril cleavage.

The final stages take place in the late nymph, after the full number of daughter fibres has formed, and after myoblast incorporation has ended. For some time fibril proliferation continues, the fibrils remaining thin, though the cross-striation is becoming more pronounced. Sections now show much evidence of mitosis among muscle nuclei (figure 239), contrasting with its paucity in earlier stages; nuclei of invading tracheal cells also show much mitosis. Eventually the fibrils thicken into sarcostyles, and with increased spacing attain the adult condition.

Owing to the faintness of the sarcolemma in fixed preparations, the time of its formation cannot readily be observed; certainly it already partially encloses the fibres before myoblast incorporation ceases.

It has come as a surprise to find that even in the male all the early stages of the myogenesis take place. Half-way through the nymphal period it normally ceases, though in some cases even discrete muscle fibres are formed in quantity. Occasionally these survive to a variable degree even in the adult insect. In other cases they soon present a markedly degenerate character, the nuclei being deeply pycnotic, and the striation faint. After the final moult only a few shreds then remain, the degenerating fibres being removed by swarms of phagocytes. The 'aberrant' muscle remains intact.

In the leg muscles of this insect, enlargement during the nymphal period takes place by multiplication of fibres—the coxal promotor muscle, for example, undergoes a four- to five-fold increase in number of its fibres—but I find no evidence of myoblast incorporation attending the cleavage. An exception must be made for the large springing muscle; here the fibre cleavage is much more intensive, and many free myoblasts are found among the proliferating fibres, into which they are absorbed. I have not examined the process in detail.

Other Cercopidae

In several other species that I have examined—*Philagra parva*, *Locris* sp., *Neoaphrophora tiegsi*—the general anatomy is much as in *Bathylus*. But in these, and in other forms (*Tomaspis* sp., *Petyllis* sp., *Anyllis* sp.), the histology is different, the fibres being coarsely fibrillated. *Bathylus* is, with respect to its wing muscles, degenerate.

In *Philagra* and *Neoaphrophora*, of which I have had a considerable range of nymphal material, development is essentially as in *Bathylus*.

General remarks on Homoptera

Although only a few families of Homoptera have so far been examined, these have already disclosed surprising differences both in the structure and in the development of the wing muscles within this single though rather diversified group. Flight, though never strong, is on a higher plane than in Orthoptera; and in the histology of the tissue this is reflected by the production of specialized fibres, sometimes with thin, sometimes with coarse (Siebold) fibrils, but always differing from the simple fibres of Orthoptera.

Common to all the species examined is the device whereby two tergal muscles are used in antagonistic capacity as depressor and levator muscles respectively. We find also, throughout the group, a feeble development of metathoracic muscles—usually because the musculature of the thorax is given over to the function of springing—abdominal muscles being occasionally called on to make good the deficiency. But beyond this it is the differences, not similarities, that obtrude. It seems that with improvement of the flying mechanism, a limit has been reached in the expediency of using individual muscles in the double capacity of leg and flying muscles, and that different families of Homoptera have reacted differently to the crisis. In cicadas, where the nymph is a sluggish subterranean creature, almost all the dorso-ventral and epipleural muscles of the mesothorax are diverted to serve exclusively the function of flight. In the Jassidae and Delphacidae, on the other hand, a simplification of the wing musculature has arisen: several dorso-ventral muscles operating on the leg base are retained as leg muscles; other muscles are lost, and of the dorso-ventral and pleural groups in the mesothorax, five at most are retained as flying muscles. The cercopids, on the other hand, retain a maximum number as pure leg muscles; but two apparently quite new wing muscles (first and second tergo-coxals) have here arisen, apparently from the tergal pro- and remotor muscles. It is probably these new muscles, and not the parent muscles, that are the equivalent of the several tergo-coxal flight muscles of Jassidae and Ricaniidae.

Regarding the histogenesis, that of cicadas is comparable with that of pure wing muscle of Orthoptera, where entire muscles arise by repeated division of a single immature nymphal fibre. The initial fibre cleavage of other groups (Cercopidae, Jassidae with multifibre number, Ricaniidae, Flatidae, Delphacidae) is a similar process; the remarkable feature that is here added to the histogenesis is the incorporation of free myoblasts into the cleaving fibre rudiments. In cercopids the myoblasts are contained within the nymphal fibres from which the wing muscles arise; in all others they are free cells, and in the growing nymph become progressively built, either singly or in chains, into the cleavage

products of an initial fibre rudiment that forms from one or more myoblasts. Finally, in Jassidae with small fibre number, the cleavage process is entirely suppressed. The general feature of this type of myogenesis is that the myoblasts bring to the growing fibre additional fibrillar, sarcoplasmic and nuclear material, and that the added fibrils accommodate their cross-striation to the pattern already existing in the growing fibre. Lack of information from other orders of insects enjoins caution in assigning to the Homoptera a central position in the evolution of the wing musculature; but certainly even the few representatives so far examined seem to make an intelligible gradation from the simple orthopteran myogenesis by cleavage, to the strange type found in higher insects, where the whole process, still further modified, is deferred till metamorphosis. Other groups of Homoptera are likely to produce additional facts on this point: in the psyllids, on which I hope to report in a later paper, cross-striation of the growing fibres does not seem to develop until the entire fibril content has formed, giving yet another gradation to the higher type of myogenesis.

In other respects the musculature of Homoptera recalls that of lower types of insects. The innervation, in the few cases examined, is at localized Doyère endings, as in Orthoptera. To direct electrical stimulation the muscles always give a good response, even when the fibrils are of the coarse (Siebold) type. In Heteroptera also, with coarse fibrils, I find that the muscle responds readily to direct stimulation.

DIPTERA

The lower members of this group, such as the tipulids, mycetophilids and mosquitoes, are not endowed with any exceptional power of flight; but in other families—Bombyliidae, Tachinidae, Syrphidae, Tabanidae and Nemestrinidae—it is strongly developed, attaining in some species, the summit of its perfection among the insects; even the hovering bees are eclipsed by the dazzling performances of some bombyliid and nemestrinid flies.

(1) *Myology*

In the Diptera enlargement of the wing vibrators is attended by reduction in number of their component muscles, and therefore by a general simplification of the musculature. There is, on the other hand, a very considerable elaboration of small muscles around the base of the wing, whose function it is to 'set' the wing by adjusting the position of various sclerites, and so act as steering muscles.

For detailed description I select a large tipulid *Plusiomyia olliffi*. The flight muscles are, of course, confined to the mesothorax. They comprise (figures 16, 17):

A. *Tergal muscles*

(i) *Median dorsal longitudinal muscle* (*m.d.l*), a large muscle attached behind to the phragma. It is the main wing depressor.

(ii) *Oblique tergal muscle* (*o.t*), a stout muscle attached below to the phragma, above to the scutum; strong wing levator.

B. *Dorso-ventral muscles*

(iii) *Tergo-sternal muscle* (*t.s*), a large muscle attached above to prescutum, below to basisternum; strong wing levator.

(iv) *Tergo-coxal muscle* (*t.cx*), arising from scutum, and attached below to meron of coxa, which has itself separated from the latter.

C. *Pleural muscle*

(v) *Subalar muscle* (*sub*) (figure 17), lateral to (iv); arises from meron, and is attached above to subalar sclerite; 'direct' wing depressor.

There is no member of the basalar group.

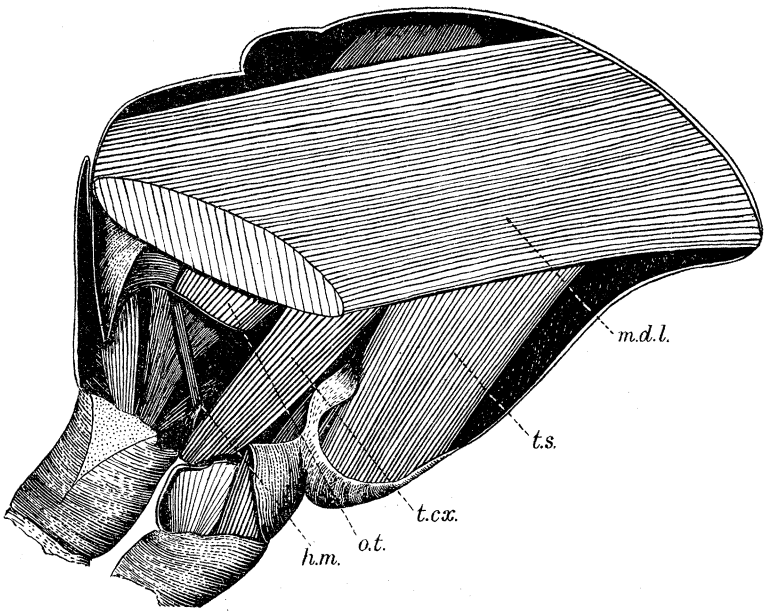


FIGURE 16. *Plusiomyia olliffi*; median bisection.

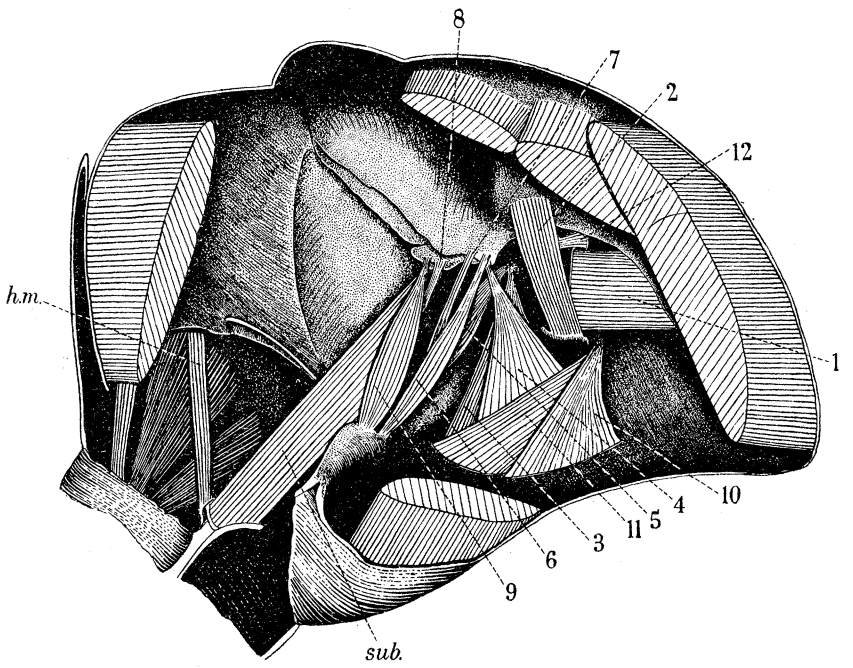


FIGURE 17. *Plusiomyia olliffi*; dissection showing laterally placed muscles, mostly wing-adjustor muscles.

D. *Wing adjustor muscles* (figure 17)

These come into view when the wing vibrators are removed. Though individually small, they are collectively of considerable size and complexity. We may group them as follows: (*a*) muscles that span the wing base, with upper attachment to the tergite, by pulling on which they tilt the wing up. They comprise muscles numbered 2, 4, 6, 7 in figure 17. Nos. 6 and 7 arise from the pleural apodeme, and are readily compared with the pleuro-tergal of all lower insects. No. 4 is a broad triangular sheet of muscle, arising from episternum. No. 2 arises from the upper end of the latter, and may be the equivalent of a muscle in Homoptera arising from basalar sclerite (description above); (*b*) muscles attached just below wing base (nos. 3, 5); by pulling in the wing articulation they must tilt the wing down; (*c*) axillary muscles, attached directly to wing base (nos. 8, 9, 12); nos. 8 and 9 arise from the pleural apodeme, and are attached to the allula; they seem to rotate the wing, tilting the costal margin up; no. 12, very small, is attached to the first axillary sclerite, and seems to extend the wing; (*d*) a muscle (no. 1), passing from pre-scutum to a pre-alar sclerite; it seems to extend the wing; (*e*) two large episternal muscles (10, 11); by pulling on the soft chitin in front of wing, they possibly tilt the costal margin down. These muscles, of course, all lack the special histology of the wing vibrators.

In the metathorax the only muscle of note is the delicate muscle (*h.m*) acting on the haltere, and arising below from the spina.

Some of the leg muscles of both segments are shown (unlabelled) in figures 16, 17; they are no longer relevant to the discussion, because not a single dorso-ventral muscle operates on the leg. The tergal wall of the mesothorax has thus become a pure alinotum, given over exclusively to flight. The extrinsic leg muscles in the mesothorax arise exclusively from the pleural ridge.

General remarks on Myology of Diptera

Apart from the more massive development in stronger fliers, two departures from the tipulid condition need mention: (*a*) the subalar muscle, found also in Culicidae and Chironomidae, is absent in most families that I have examined (Tabanidae, Nemestrinidae, Muscidae, Syrphidae, Bombyliidae, Tachinidae, Therevidae, Asilidae, Drosophilidae). Of the complex group of muscles that initiated flight in the Orthoptera, only four have thus survived; (*b*) in many families a large tergo-trochanteral muscle is present—Drosophilidae (Williams & Williams 1943), Tabanidae, Therevidae, Leptidae, Muscidae, Tachinidae, Syrphidae—but not Bombyliidae, Stratiomyiidae, Chironomidae or Asilidae.

(2) *Histology*

In the structure of the wing vibrators, the Diptera display remarkable features that do not seem to have been noticed hitherto. The system of internal tracheae is often highly organized, in a way that we do not find in other insects—I have, for instance, found nothing similar in any Hymenoptera. This can confer on the fibres a singular complexity of structure and enables them, in exceptional cases, to attain dimensions that are truly remarkable. Two examples, selected to show extremes of fibre type, will first be given.

(*a*) In *Neoaratus hercules*, a large asilid capable of flying with great speed and power, the longitudinal muscle, which is very massive, consists of about 230 narrow fibres, measuring

0·12 to 0·17 mm in thickness (figure 251, plate 31); the tergo-sternal consists of about 110 such fibres; the tergo-coxal of 120; the oblique tergal of about 60. In another much smaller asilid (*Neoitamus rudis*) the fibres are smaller and less numerous; the longitudinal, for instance, has about 80 fibres (figure 252).

(*b*) In *Rutilia potina*, a large and very active dexid fly, the thorax approaches in size to that of *Neoaratus hercules*, the flight muscles being only a little smaller. The longitudinal muscle here consists of only six fibres (figure 261), but they are of giant dimensions (the attached scale represents 1 mm); the first tergo-sternal consists of three such fibres, the other two muscles of two each. This fibre number is constant throughout the tachinid-muscid-anthomyiid group of flies, and in others described below, and in the case of all the larger species leads to giant fibre formation. Examples are shown in figures 265 to 268, plate 31; they relate to *Fannia canicularis* (Anthomyiidae), *Stomoxys calcitrans* (Muscidae), *Thelaira* sp. (Tachinidae), *Calliphora stygia* (Tachinidae). All are to the same scale as figure 261.

We thus distinguish in the Diptera, according to their fibre pattern, two types of musculature: in the one the fibres are narrow and of no fixed number even among individuals of a single species (e.g. in the stratiomyiid *Neoexaireta spinigera* the longitudinal muscle contains from fifteen to over thirty fibres according to the size of the individual, but the diameter range of the fibres is small); in the second group the fibre number is constant, but they vary in size, even within a single species (e.g. in *Lucilia sericata*, with six fibres in the longitudinal muscle, the area of cross-section of a single fibre in large individuals is more than double that in small). There is, however, a small but important group of flies (Syrphidae, Bombyliidae) to whom the above statements do not apply; here the fibre number is small but variable, not only in different species, but even in individuals of a single species. In the longitudinal muscles the fibre number lies mostly in the range 5 to 9. The variation within a single species may be illustrated by reference to *Syrphus viridiceps*: in large individuals there are up to nine fibres, in small only six; figure 258 shows a cross-section with seven on one side, eight on the other.

Giant fibres might seem incompatible with efficient oxygenation, and bring with them the problem of nutrition of the fibres. Oxygenation is met by the development of a highly organized system of internal tracheae. The tendency of tracheae to enter the fibre in rows, to which reference has already been made in the case of Homoptera, is, in Diptera, highly developed; indeed, the penetration of the sarcolemma by sheets of tracheae would here, in many cases, be a more apt description. In some it leads to the production of deep marginal fissures between the content of sarcostyles (e.g. figure 262); in other cases these almost transect the fibre (figure 269); in yet others the cleavage into columns of sarcostyles is complete (figure 281), and these are the most misleading of all, for they simulate groups of discrete fibres. But all have in common the presence of a single investing sarcolemma, bridging but not extending into, the clefts, and this is the one criterion for assessing their status as fibres; but it is decisive. Repeated statements in the literature (see Introduction, §A) as to the absence of a sarcolemma in wing muscle are plainly often based on fibres of this type; the internal columns of sarcostyles that simulate muscle fibres (cf. figure 288) are not delimited by such a membrane; they are, however, delimited by sheets of tracheae (figure 285), and this seems to account for the statement that the sarcolemma is, in wing muscle, a web of tracheae (Holmgren).

Since some doubt* may still be felt as to the correctness of these statements (particularly in view of the remarkable innervation described below), some further comment is called for. The giant fibres of *Rutilia potina*, referred to above, measure up to 1·8 mm in width. A fragment (representing about the medial fifth) of the third (from above) longitudinal fibre is shown in figure 262. The magnification does not suffice to show the sarcolemma, but this is shown in figure 263, plate 31. The sarcostyle content is deeply incised by narrow fissures that grow in from the margin of the fibre, but into these the sarcolemma does not grow; nor is the regularity of the sarcostyle distribution in the interior of the fibre upset by possible unstained sarcolemmal sheaths. Within the clefts lie the muscle nuclei and tracheae. The latter are, of course, only seen with selective tracheal impregnation. I have had more success in impregnating the tracheae in *Calliphora stygia*, so will illustrate the point by reference to that species. Figure 270, plate 31, shows part of a cross-section of one of its giant fibres; note the large tracheae in the clefts, and the continuous delicate network pervading the whole interior of the fibre. Can there be any doubt that the giant fibres are single muscle fibres?

The building of giant fibres in the Diptera is so closely bound up with elaboration of their internal tracheae, that it will be helpful, at this stage, to describe briefly the general character of the latter. I select, as an instructive example, a small sapromyzid fly *Poecilohetaerus schineri*. There are six fibres in the longitudinal muscle, but the insect is too small to permit giant fibre formation. The entering tracheae, without spiral intima, fan out in narrow clefts between large groups of sarcostyles (to which clefts, also, the muscle nuclei are confined), and there merge with adjacent tracheae to form a continuous net of air-filled vessels; in figure 282, plate 32, for instance, the section passes above along such a cleft, so that the entire tracheal net is seen even without change of focus. The larger tracheae, in this insect, are not restricted to the marginal zone of the fibre, but actually cleave the fibre into polygonal columns of sarcostyles, in cross-section easily mistaken for single thin fibres (figure 281). Sometimes by chance a longitudinal section will be in the plane of a trachea-filled cleft extending almost across the fibre. Figure 283 is an example; it shows the closed tracheal net, almost in a single focal plane, for only in a few places are some of the tracheae unfocused. From this closed net occasional tracheae enter the columns of sarcostyles, along which they run some distance before re-entering the main tracheal net; one such trachea is shown for its complete length in figure 282 (asterisk).

In the larger and more active Diptera this simple tracheal pattern is much elaborated, and in the most active species undergoes a truly remarkable development. A pattern of moderate complexity is found in the house-fly. There are six fibres in the longitudinal muscle, and the size of the insect permits incipient giant fibre formation. A tracheal impregnation of the fourth fibre is shown in figure 284; note how the larger tracheae are confined to clefts between the columns of sarcostyles; note also entrance of tracheae into the columns in numbers far greater than in the sapromyzid. Longitudinal sections that pass along a cleft show the sheet of large entering tracheae; note in figure 285 how the

* *Added in proof*, 8 *November* 1954: In a paper that I have overlooked, Partmann has actually described these muscles, but apparently without recognizing them as single giant fibres. See Partmann, W. 1948 *Zool. Jb.* (*Anat. Ontog*) **49**, 506.

larger tracheae lie almost in a single plane, for they do not leave the plane of focus; note also a small amount of anastomosis between the finer branches of the entering tracheae. Figure 286 is a longitudinal section but so directed that it transects the clefts lengthwise (i.e. it is at a right angle to the former); note how the branches from the rows of tracheae turn to enter the columns of sarcostyles, where they branch to form a continuous net by fusing with tracheae from the adjacent row. Finally figure 287, which is a fragment of a cross-section, shows the degree of intimacy of the tracheae to the sarcostyles. Note, incidentally, that the muscle nuclei are confined to the clefts (figures 285, 286).

The climax of this type of tracheation is reached in some of the syrphid and nemestrinid flies. I select for description the nemestrinid *Trichophthalma punctata*. This is a hovering species that can remain for minutes poised motionless in the air, the high-pitched whine of the rapidly vibrating wings first drawing attention to the presence of the insect; yet when disturbed can flash off with a speed that the eye can scarcely follow. The clefts, in which lie some of the muscle nuclei, are here confined to the margin of the fibre. The entering tracheae are surprisingly large (figure 292); they fan out, as usual, from their point of entrance, but undergo only a small amount of anastomosis with fine branches of adjacent tracheae. The system of fine vessels which then carries the air to the sarcostyles is, in this insect, related to the pattern of cross-striation of the fibre. Sections directed along the fibre in such a way as to intersect the clefts lengthwise, give the picture shown in figure 293; we see here, as in the house-fly, how the fine branches emerge from the clefts to enter the columns of sarcostyles, across which they pass to merge with the tracheae of the adjacent row; but in this case they run a transverse path, two to each cross-striation. The middle of the fibre, which is not cleft, is supplied by similarly spaced tracheae that pass directly across the fibre (figure 292). The picture is completed by reference to the cross-section (figure 294): this shows the branching of the fine vessels in a plane at right angles to the long axis of the fibre; it is a much finer network than in the house-fly, there being hardly a sarcostyle that is not in contact with, or even encircled by, a fine trachea. The reader will understand, by comparison with figure 293, that the picture shown in the cross-section is repeated, at intervals of half a cross-striation, along the whole length of the fibre.

I give now a brief statement on the fibre pattern in the main families of Diptera.

Agromyzidae, *Sapromyzidae*, *Scatopsidae*, *Drosophilidae*. In all species examined the fibres are few (six in longitudinal muscle) (figure 264, plate 31); but there is little opportunity for fibre enlargement, for they are mostly small insects. Exception must be made for some of the larger sapromyzids, in which fibres 0·4 mm in width occur. The tracheal pattern in all species examined is of the type described above for *Poecilohetaerus schineri*.

Rhyphidae, *Cordyluridae*, *Empydidae*, *Dolycopodidae*. Here also we find only six fibres in the longitudinal muscle; but the insects are never large, and do not give scope for giant fibre formation. In *Tapeigaster* sp. (Cordyluridae), which is the largest that I have examined, the fibres measure 0·4 mm across. The tracheal pattern is as in the foregoing families, but stronger developed.

Therevidae, *Leptidae*. There are six fibres in the longitudinal muscle, rectangular in cross-section; the clefts are confined to the margin of the fibre. In the therevid *Ectinorrhynchus brunneus*, where alone I have examined the tracheae, these enter in deep marginal clefts;

there is a fairly dense tracheal net among the sarcostyles, with a tendency of the vessels to run longitudinally. The fibres, in this species, are $0{\cdot}35 \times 0{\cdot}2$ mm in cross-section.

Tipulidae. Here the fibres are thin and numerous. Figure 256, plate 31, shows a cross-section through *Macromastix clarkiana*, a medium-sized species, and this fibre pattern is general for the group. The range of fibre number and size is considerable: in the small *Limnophila incisuralis* there are twenty fibres in the longitudinal muscle, about 0·05 mm in diameter; in the large *Plusiomyia olliffi* there are thirty to thirty-five fibres, up to 0·1 mm thick. In the very small species, with feeble flight, the tracheation is poor; the vessels enter in rows, but they are widely spaced, and do not therefore cleave the fibre content. Figure 291, plate 32, shows the extreme paucity of tracheae in *Limnophila incisuralis*. In larger species the tracheal supply is richer. The tracheae enter at smaller intervals, and therefore produce some marginal cleavage in the fibre. The pattern for *Macromastix clarkiana* is shown in figure 271, plate 31; note how the tracheae enter the section in rows.

The muscle nuclei are scattered at random in these fibres.

Chironomidae. The fibres are few, but not constant in number. *Chironomus duplex* has six in the longitudinal muscle; another (undetermined) species has five. The insects are too small to permit giant fibre production.

Culicidae. In all species examined each longitudinal muscle consists of two tiers of six fibres each. This fibre arrangement seems to be quite inflexible, for both small and large species conform. Even in the large *Mucidus alternans*, the fibres measure no more than 0·17 mm.

The tracheae enter at random along the fibre. The finer tracheae run a predominantly longitudinal course (figure 295, plate 32).

Psychodidae. Despite the small size of the insects, the fibres are numerous (figure 255, plate 31). The longitudinal muscle comprises about thirty fibres, measuring under 0·03 mm, being therefore among the thinnest in Diptera. The tracheae run mainly longitudinally, without pattern.

Stratiomyiidae. Here the fibres are thin, and in the larger species numerous. In *Neoexaireta spinigera*, with a maximum of thirty fibres, the latter are cleft into columns of sarcostyles, as in sapromyzids. Within the columns the tracheae are abundant and run mainly lengthwise.

Asilidae. The smaller species are weak and slender insects; the largest contain the giants among the Diptera. In the huge and fearsome *Phellus glaucus* there are about 450 fibres in the longitudinal muscle, about 0·15 mm thick; in the delicate *Leptogaster* there are about twenty fibres, about 0·07 mm thick. The fibre pattern of the muscle is shown in figures 251, 252, plate 31.

The tracheal pattern is surprisingly similar to that of tipulids, widely spaced tracheae entering in rows, but without affecting the sarcostyle pattern; the finer tracheae run mostly lengthwise between the sarcostyles. Figure 273, plate 31, shows a preparation from a small species (*Neoitamus rudis*); note the entering tracheae on the left, undergoing anastomosis; note on the right the longitudinal path of tracheae that enter the section 'from below'.

This simple type of tracheation in a predatory fly came as a surprise; but asilids, even the larger species, are not habitually 'on the wing', preferring to pounce on their victim as it passes. In the much larger *Neoaratus hercules* the pattern is similar to that of *Neoitamus*, but

denser; I have some silver preparations in which the longitudinal vessels appear connected by a system of most delicate transverse vessels, but am not sure that this is not an artifact.

Apioceridae. The fibre pattern is as in Asilidae.

Nemestrinidae. My description is confined to several species of *Trichophthalma*, including the large *T. bancrofti*, with a thorax 1 cm in width. The remarkable flying power of these insects is familiar to all students of Diptera.

The musculature is massive; the fibres are numerous—in *T. punctata* there are about thirty-five in the longitudinal muscle, in *T. bancrofti* about sixty—and are disposed as in figure 254, plate 31. They are mostly polygonal in cross-section, and in the large *T. bancrofti* some attain a width of 0·75 mm.

The tracheae have been described above. The muscle nuclei lie in the marginal clefts, or in the middle of the fibre among the sarcostyles.

Tabanidae. My description is based on numerous species of *Tabanus*, and a few species of *Diatomineura*, *Silvius*, *Erephopsis* and *Pelecorrhynchus*. The musculature is massive, and much as in nemestrinids. The fibre pattern for *Tabanus imperfectus* is shown in figure 253; in the smaller species there are only two such tiers of fibres; in the large *Pelecorrhynchus* there are four.

I have had much difficulty in examining the tracheae, for they do not impregnate well. A fragmentary impregnation of *T. edentulus* indicates a tracheal pattern very much as in nemestrinids.

Ortalidae. Here the fibres are few (six in the longitudinal muscle), and in the larger species this leads to giant fibre production; for example, in *Lamprogaster laeta* the fibres have a width of 0·6 mm. In the smaller species of *Dacus*, *Pogonortalis* and *Ceratitis* they seldom exceed 0·2 mm. The fibres are mostly rectangular in cross-section; but in *Lamprogaster* they are of irregular form, and deeply incised by large tracheae (figure 275, plate 31). In *Dacus* and *Pogonortalis* the invading tracheae tend to cleave the fibre into discrete columns of sarcostyles, as in some of the lower families above described.

Muscidae, Anthomyiidae, Tachinidae. These closely related families may conveniently be considered together. In all cases the fibres are few (six in the longitudinal muscle); and in all the larger species this leads to the production of giant and often complex fibres. In the smaller forms like muscids and anthomyiids, the fibres tend to a rectangular cross-section (figure 266, plate 31); in the larger calliphorines and dexids they are considerably widened, and in places are deeply indented by invading tracheae (figures 261, 268). The following gives the maximum fibre width in a few species: MUSCIDAE: *Musca domestica*, 0·35 mm; *Muscina stabulans*, 0·5 mm; ANTHOMYIIDAE: *Helina victoria*, 0·5 mm; TACHINIDAE: *Tritaxys heterocera*, 0·9 mm; *Sarcophaga* sp., 1 mm; *Calliphora stygia* (figures 268, 269), 1 mm; *C. nigrithorax*, 1·2 mm; *Rutilia potina* (figure 261), 1·8 mm.

The tracheation is of the type already described for the house-fly; tracheae enter the fibres in sheets, which either completely or incompletely cleave the fibre into groups of sarcostyles, adjacent rows of tracheae being connected by fine vessels that traverse the sarcostyle columns. Many of the calliphoras and rutilias are active boisterous insects, and the tracheal net is therefore much richer than in the house-fly; but in no case is there any relationship to the cross-striation of the fibre. The nuclei, in all cases, lie in the clefts between the sarcostyles.

Syrphidae. My description is based on various species of *Syrphus*, *Eristalis*, *Chrysogaster*, *Helophilus* and *Eumerus*.

The fibre number is small but variable, even in members of a single species. In all individuals of *Eristalis* that I have examined there are eight fibres in the longitudinal muscle, in *Chrysogaster*, *Helophilus* and *Eumerus* six; in *Syrphus viridiceps* six to nine. In large flies like *Eristalis* some fibres attain a width of 0·75 mm.

In most species the fibres are rectangular in cross-section; in *Eristalis* and *Helophilus* they are deeply grooved by large longitudinally running tracheae (figure 274, plate 31).

In *Syrphus viridiceps* the tracheal pattern is similar to that of muscids. In *Eristalis*, on the other hand, the fine tracheae are orientated with respect to the cross-striation; the pattern here is very similar to that of nemestrinids, the dense network of fine vessels expanding in a plane at right angles to the long axis of the fibre, and being repeated at intervals of half a cross-striation. The narrow clefts in which the entering tracheae lie are readily seen in figure 274.

Bombyliidae. My description is based on species of *Comptosia*, *Bombylius*, *Lomatia*, *Phthiria*, *Villa*, *Systropus*, *Systoechus* and *Sisyromyia*. All are strong fliers, particularly *Sisyromyia*, which emits a high-pitched whine during flight. The muscle fibres are few, and therefore in most species large. In the species of *Phthiria*, *Bombylius*, *Systropus* and *Comptosia* that I have examined, there are usually five fibres in the longitudinal muscle (*Comptosia extensa* has seven or eight). In *Villa* there are six or seven, in *Systoechus* nine, some attaining a width of 1 mm. In *Sisyromyia aurata* the specimen shown in figure 259, plate 31, has fifteen on one side, twelve on the other, the largest over 1 mm wide. In both these genera and in *Comptosia* there is another group of fibres above the longitudinal muscle, with anterior attachment midway along the segment.

In *Sisyromyia aurata* the tracheae lie in marginal clefts that do not reach the middle of the fibre (figure 260); the net of fine tracheae that arises from the entering vessels is very dense, as expected, but surprisingly shows no relationship to the cross-striation.

Oestridae. The fibre pattern in this small group has nothing in common with that of muscids, to which the family is said to be allied.

In *Gastrophilus intestinalis* the wing muscles consist of narrow fibres, ungrouped, a cross-section recalling asilid muscle. The tracheal pattern is shown in figure 272; the entering tracheae are rather widely spaced, and do not cleave the fibre into sarcostyle columns; the branches expand into tracheal nets disposed at right angles to the long axis of the fibre, and repeated along the fibre at regular intervals of a single cross-striation (the transverse tracheae in figure 272 represent a section cut across the tracheal net). In *Hypoderma bovis* there are about thirty fibres in the longitudinal muscle. In *Oestrus ovis* each longitudinal muscle consists of six separate muscles, each comprising twelve to twenty bundles of extremely narrow fibres (figure 257).

Other histological features

Throughout the Diptera the wing vibrators, and, in the metathorax, the muscle to the haltere, fall into the category of coarsely fibrillar (Siebold) muscle. In most species the fresh sarcostyles measure only a little over 2μ thick, i.e. rather less than in Hymenoptera; *Neoitamus rudis* (Asilidae) is exceptional, the sarcostyles being about 3·5μ thick (figure 276,

plate 32), while in *Drosophila* they are unusually thin (1·6μ, figure 279). The inter-*Z* distance ranges from 3μ to a little over 4μ, and this at once distinguishes them from the sarcostyles of Hymenoptera, where the inter-*Z* distance is less than 3μ. Chironomids and mosquitoes are, however, exceptional, the inter-*Z* distance being as little as 2·0 to 2·3μ (figure 277). *Zf*- and *Mf*-disks are usually visible even in the fresh sarcostyle (figures 278, 279), and are accentuated by phase contrast; yet in some species they are quite invisible in the fresh sarcostyle (figures 276, 277), but can be made evident by staining (figure 280). An unusual feature met with in Diptera, and already noted by Kölliker, is the faintness, and frequent invisibility, of the *Q*-band. To fixation, the sarcostyles are very refractory, and only the most exceptional preparation gives even a hint of their composite structure (figure 280); experience gained exclusively from Diptera would hardly have given a clue to the real nature of the composite sarcostyle of wing muscle.

The cross-membranes also are refractory to present methods; only in exceptional preparations have I seen *Zs*, whose presence we infer from the cross-alinement; *Ms* has never appeared in my preparations.

Sarcosomes are abundant, being transversely aligned to either side of the *Z*-membrane.

Innervation

I have had available some excellent preparations made for me by Mr Willis with his silver method. They relate to *Drosophila*, *Musca domestica*, *Fannia canicularis*, *Eristalis tenax* and *Rutulia potina*. It is at once evident in these preparations that the innervation is quite different from that of the types so far described. Doyère endings do not occur, the nerve fibre passing deep into the muscle fibre. Here it undergoes a small but variable amount of branching, being always confined to the zones of sarcoplasm between the columns of sarcostyles. Figure 288 shows such an ending in *Musca domestica*; the branching is far more extensive than the single section can show, and has been completed (dotted line) from the two adjacent sections. Figure 290 shows an intracellular ending from *Drosophila*.

A second feature of the innervation is that it is not localized, but that there are numerous such endings all along the fibre. In *Drosophila* each muscle fibre is attended by a nerve that runs along it for almost its entire length, delivering, at short intervals, fine branches to the muscle fibre. The anterior end (about a fifth) of one of the muscle fibres, with attendant nerve, is shown in figure 289; the entering nerve twigs are indicated by asterisks. At least twenty-four such nerve filaments can, in this preparation, be seen entering the muscle fibre at short intervals along its length. The nerve that attends each muscle fibre appears to be not a single coarse nerve axon, but a closely knit bundle of very delicate fibres. This becomes apparent when we retrace it, in serial section, into the thoracic ganglion, where it becomes splayed out into most delicate fibrils arising separately from the fibrillar substance of the ganglion.

We gain the impression that this multiple innervation is a device for throwing the whole muscle fibre instantaneously into contraction, by starting the contraction at many points along its length; but in view of the remarkable observations of Pringle (1949) that frequency of stimulation falls far short of frequency of contraction, no decision can at present be reached on this point.

(3) *Development*

My observations are confined to *Drosophila*. The wing-muscle fibres, of which there are six in the longitudinal muscle, are rectangular to oval in cross-section, with a maximum width of 0·1 mm. The sarcostyles are grouped into columns, twelve to twenty-five per fibre. In the sarcoplasm between the columns lie most of the nuclei; the others are just under the sarcolemma.

For present purposes much importance attaches to the nucleus:sarcostyle ratio. In thick (15μ) serial sections, stained with Ehrlich's haematoxylin, the nuclei can be counted with considerable precision; the sarcostyles I have counted from photographs of cross-sections. The counts, which are subject to only slight error, point to a 1:1 ratio:

	nuclei	sarcostyles
3rd fibre (from above)	2321	2361
4th fibre	2326	2345
6th fibre	2062	2051

Development starts at the beginning of pupation, the wing muscles arising out of free myoblasts which, in the adult larva, are found adhering in small numbers to the outer surface of certain larval muscle fibres in the mesothorax; the dorsal longitudinal muscle, for example, develops from myoblasts associated with three fibres of the corresponding larval muscle. In the young pupa the myoblasts multiply actively, spreading out over the surface of the muscle fibres, which themselves are now degenerating (figure 240, plate 30). Leucocytes do not play a part in removal of the degenerated larval tissue.

Soon the myoblasts from adjacent fibres merge to form a common envelopment for all three fibres; at the same time some of the myoblasts begin to invade the fibres themselves (figure 241). By the end of the first day (20° C) the three larval fibres have been almost completely replaced by three compact columns of myoblasts, around which other myoblasts swarm (figure 242); vestiges of the larval fibres survive only at the tips of the myoblast columns, serving to attach the muscle rudiment to the epidermis. Continuing to multiply, the myoblasts, early in the second day, form a thick sheet of cells lying just below the roof of the segment (figure 243). Their component cells are now quite irregularly disposed, the former disposition into columns having apparently no significance in myogenesis.

In pupae that are only slightly older, the rudiments of the wing-muscle fibres are making their appearance. For example figure 244, which is a cross-section at the same level as in figure 243, shows the two developing fibres of the oblique tergal muscle, and the six fibres of the developing dorsal longitudinal muscle. The myoblast columns from which the young fibres have arisen are seen in figure 243. Under higher magnification each developing rudiment is seen to consist of a column of most delicate fibrils, which enclose a core of nucleated sarcoplasm, and around each developing fibre is a swarm of free myoblasts.

I have had much difficulty in directly observing the processes that have led to the formation of the young fibres. The following account is given with respect to the tergosternal muscle. The rudiment of this muscle lies immediately alongside the lateral thoracic wall, and is at first a thin column of spindly cells, which tapers to its attachment above on the thoracic wall (figure 248). Considerable enlargement takes place early in the second day, and within the column of densely crowded myoblasts bundles of most delicate fibrils

traversing the full length of the myoblast column are then for the first time visible. Figure 249 shows a typical longitudinal section; the asterisk indicates a fragment of a fibril bundle, while to the left (two asterisks) is a much thinner bundle, comprising only a few unresolved fibrils. We have to determine how the fibrils have in the first place arisen, and how the bundles grow to form the adult fibre. Since the muscle rudiment is a column of closely crowded but discrete cells, we can hardly escape the conviction that the fibrils must be outgrowths of individual myoblasts; but they are, in *Drosophila*, of such extreme delicacy and faintness, and the myoblasts are so densely crowded, that even in the best preparations it is usually impossible to follow separate fibrils for any considerable distance.

In slightly earlier pupae we get indications as to how the fibril bundles arise; instead of bundles we see, here and there, fibrils of the utmost delicacy passing some distance along the myoblast column. Figure 250 is an attempt to photograph them, at single focus; the asterisk here points to a myoblast which has prolonged into a most delicate filament, which is soon accompanied by a second derived from another myoblast (two asterisks). Not infrequently, in later pupae, we see individual myoblasts prolonging into delicate filaments alongside the fibril bundles, but in no case can they be followed for any distance through the densely packed myoblast column.

Plainly we are confronted here with the problem that we have already met in various Homoptera, in which the fibre is gradually built up by progressive addition of free myoblasts to the fibril column; but in *Drosophila* the tissue is even less amenable to observation. The nucleus:sarcostyle ratio above given is very suggestive, and points to the probability that each myoblast generates a single fibril, bringing with it its nucleus and sarcoplasm; but for the present this is an inference only.

Before the middle of the second day the young muscle fibres have become fairly well defined within the former myoblast column. The peripheral zone of fibrils, enclosing an axial core of sarcoplasm to which the nuclei are confined, and to which reference has already been made above, are now apparent. The whole muscle rudiment then falls apart into its constituent fibres (three in the tergo-sternal muscle), each still invested by a swarm of myoblasts; figure 245 shows, for example, the six fibres of the longitudinal muscle. There is very little to distinguish the nuclei of the young muscle fibres from the surrounding myoblasts except the thin investment of cytoplasm that the latter carry, and even this is not regularly seen in cross-section if the myoblasts are longitudinally orientated.

By about the middle of the second day myoblast incorporation is completed. The peripheral zone of fibrils has now broken up into a variable number of sub-columns, between which some of the sarcoplasm has insinuated itself (figure 246); of the fibril columns some have moved into the interior of the fibre. These events are attended by a temporary shortening and thickening of the muscle rudiment, which becomes reduced to a third its initial length, the tonofibrillae that attach it to the epidermis being drawn out into long tenuous filaments that suspend the muscle in the thoracic cavity.

In later pupae the fibres gradually revert to their initial length; the fibrils thicken and become more widely spaced; the nuclei are still very large; a sarcolemma is now plainly seen (figure 247).

Up to the completion of myoblast incorporation, the fibrils are without recognizable cross-striation; the latter appears during the third day of development.

General remarks on Diptera

High specialization of the thoracic musculature is here everywhere in evidence. Reduction of the originally complex group of muscles, eventually to a few essential muscles operating on a pure alinotum, is remarkable, as is also the tendency to simplification of fibre pattern leading to the production of a few fibres only, but often of giant dimensions. Restriction of the ability of the sarcostyles to shorten, and innervation of single fibres by many nerves that penetrate deeply among the sarcostyles, and without Doyère endings, are other remarkable features, as is also the strange lack of synchronization between frequency of motor discharge and fibre response (Pringle). The only histogenesis so far examined, that of *Drosophila*, is plainly a specialization of that described above in Homoptera, the entire process being relegated to the period of metamorphosis.

Conclusion

The reader will feel some surprise at the undue emphasis that has been placed above on certain insect types, to the complete exclusion of other often better known groups. This restriction, not originally intended, became a necessity once the diversity of the myogenesis in the different groups was discovered.

The Orthoptera were selected for detailed study because of the intelligible picture they give of the early adaptation of the thoracic musculature to the new function of flight. The Diptera, on the other hand, are, with respect to their flying musculature, perhaps the farthest removed from the primitive insects, and reveal the flying mechanism at the summit of its achievement. In the Homoptera the wing musculature appears to be in a state transitional between the two, not only in its gross morphology, but also in its histology and histogenesis, and seemed therefore likely to give instructive information in regard to the evolution of the higher type of musculature. These were the considerations that determined my choice of material.

The results are fully in accord with orthodox opinion that even the most specialized musculature has been derived from the archaic musculature of lower insects; and although much new material will be found in the above account, there is, on this point, nothing basically new.

Orthodox opinion that the flying muscles are composed of fibres that are strictly comparable with those of normal trunk muscle is also confirmed. But in this instance it would be difficult to justify current opinion on the evidence hitherto available. There is no generally accepted theory of the muscle fibre, for on almost every point authorities differ. This entailed, at the outset, a critical examination of all the contentious points regarding the structure of the 'normal' arthropod fibre. The result of this examination forms the introduction to the present paper; in particular, the recognition of the compound sarcostyle has been of crucial importance, for it has shown the real meaning of the coarse fibrils in wing muscle, where the very reverse of coarse fibrils might have been expected. Evolution of the wing musculature, it now appears, is attended by a progressive adaptation of the muscle fibre to high-frequency isometric contraction; and in the Diptera, where this reaches its peak, the muscle fibres are changed almost out of recognition.

In the histogenesis of the wing musculature, the most noteworthy finding is its diversity in different insect types. In the Orthoptera the muscles enlarge by fibre cleavage, and only near the last moult, when the muscle prepares to accept the additional role of a flight muscle, do its fibres undergo the small structural changes that adapt them to the purpose. In the highest insects the wing muscles are produced at metamorphosis by the co-operation of multitudes of myoblasts, the scanty data indicating that each myoblast in the process generates a single sarcostyle. The interest of the Homoptera lies in the fact that within this single group a diversity of myogeneses occurs, free myoblasts co-operating in various ways with the cleavage products of rudimentary fibres, to generate the wing-muscle fibres, and that in this diversity there is an intelligible gradation to the simpler development of Orthoptera. But there is no implication that other intermediate orders might not show similar gradations. Indeed in Neuroptera, on which I hope to report later, there is a peculiar form of fibre cleavage, in which single hollow rudimentary fibres, enclosing a core of free myoblasts, undergo limited cleavage, the myoblasts adding themselves in chains to the cleavage products; and in ephemerids, also, there is a most intense fibre proliferation, with numerous myoblasts involved, but here they do not seem to generate fibrils. The student of morphogenesis will derive much interest from the phenomenon of myoblast incorporation; its significance is at present obscure.

The most interesting general point that emerges is the remarkable and hitherto unsuspected plasticity in the process of myogenesis, so that basically similar fibres are formed by what are apparently quite dissimilar histogenetic processes. To what extent muscles other than the wing muscles show diversity of histogenesis is not known.

I wish, in conclusion, to express my thanks to the entomologists of the National Museum, Melbourne; of the South Australian Museum, Adelaide; of the Commonwealth Scientific and Industrial Research Organization, Canberra; and to Dr F. H. Drummond of the Department of Zoology, Melbourne University, who have helped me greatly by the identification of material. Dr K. H. L. Key (Canberra) and Mr A. F. O'Farrell and Dr A. Stock (Armidale) sent me the *Chortoicetes* and blattid material respectively on which the above account is based. To Mr J. A. Thomson I owe the developmental stages of *Acridopeza*, while to Dr J. W. Evans I am indebted for a supply of various preserved Homoptera. I am much indebted to Mr A. G. Willis (Zoology Department, Melbourne University) for critical reading of much of the above manuscript; to him also I am indebted for the beautiful silver preparations on which much of the above account of innervation is based. Finally, I wish to express my thanks to Mr E. Matthaei, of the Optical Laboratory, Melbourne University, for the skill with which he has prepared the numerous photographs.

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DESCRIPTION OF PLATES 17 TO 32

Note. The attached scales of magnifications are, unless otherwise indicated, a Grayson ruling, in which a single subdivision is about 1μ.

Contractions employed: Alc.B., alcoholic Bouin fixative; A.A.T., alcohol-acetic-trichloracetic fixative; I.H., Heidenhain's iron-alum haematoxylin.

PLATE 17

FIGURES 18 to 24. Leg muscle; rat.

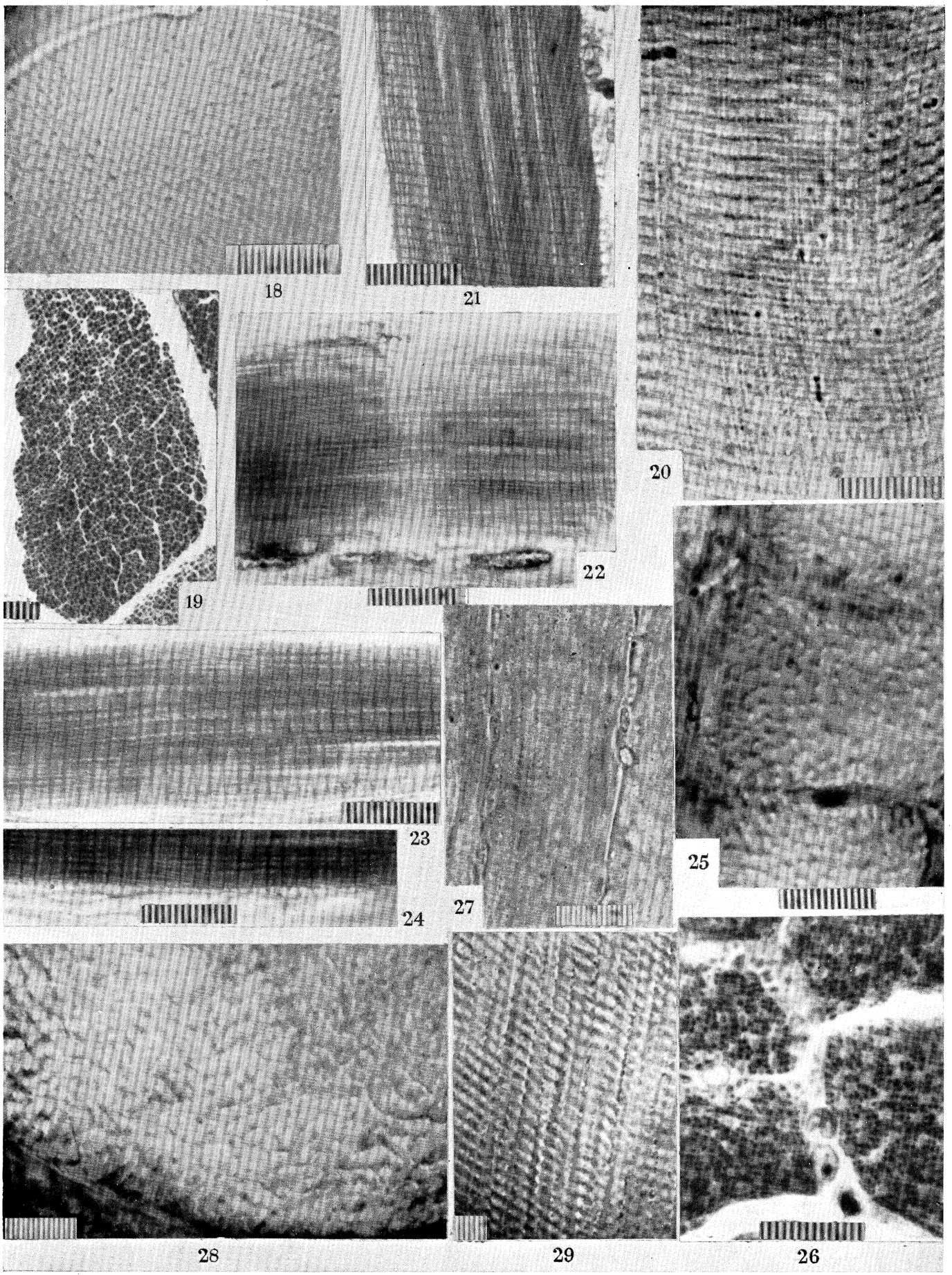
18. Cross-section, showing Cohnheim's areas; fresh frozen section.
19. Cross-section, showing fibrils. Alcohol trichloracetic, I.H.
20. Longitudinal section, showing Kölliker's columns; fresh frozen section.
21. Longitudinal section, showing fibrils and *Z*-membranes. Alcohol trichloracetic, I.H.
22. Longitudinal section, showing *Z*- and *M*-membranes. Apathy's alcohol-corrosive fixative, Heidenhain's thiazine red, methylene blue stain.
23. Similar preparation, showing two 'vernier effects' of cross-membranes.
24. Fragment of similar preparation, showing cross-membrane attachment to sarcolemma.

FIGURES 25 to 27. Pectoralis major (flying) muscle of finch (*Zonaeginthus oculatus*).

25. Cross-section, showing Cohnheim areas; fresh frozen section.
26. Cross-section, showing fibril pattern. Alcohol trichloracetic, I.H.
27. Longitudinal section, showing Kölliker's columns, with sarcosomes in interstitial substance; fresh frozen section.

FIGURES 28, 29. Femoral muscle, plague locust (*Chortoicetes terminifera*).

28. Cross-section, showing Cohnheim pattern; fresh frozen section.
29. Longitudinal section, showing Kölliker's columns and sarcosomes; fresh frozen section.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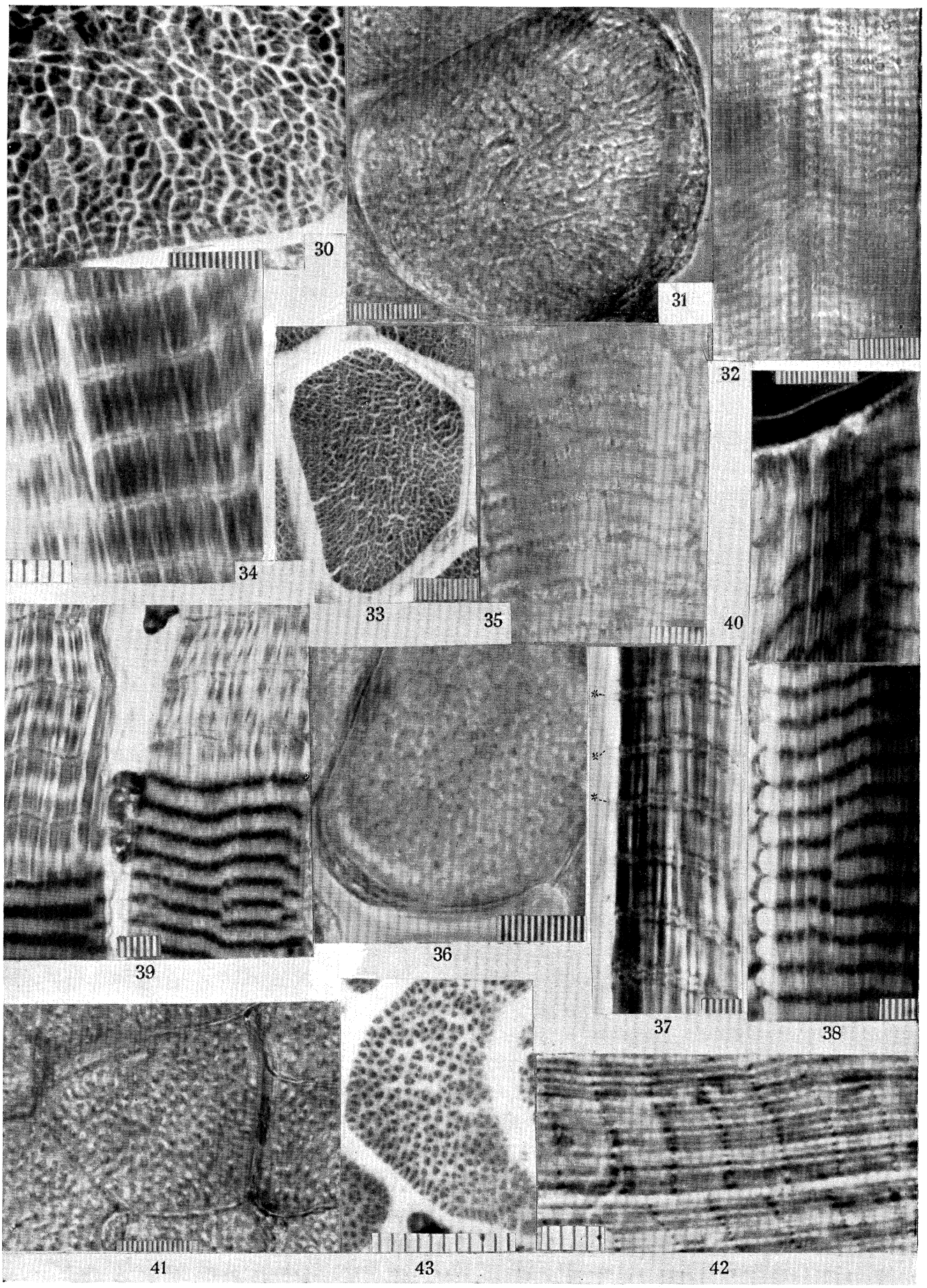
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PLATE 18

FIGURE 30. Femoral muscle, *Chortoicetes terminifera*; cross-section showing sarcostyles. A.A.T., I.H.

FIGURES 31 to 34. Leg muscle, scarab larva (*Aphodius howitti*).

31. Cross-section; fresh frozen section.
32. Living muscle fibre, seen in optical section through transparent leg.
33. Cross-section showing sarcostyle pattern. Alc.B., I.H.
34. Longitudinal section, showing sarcostyles. Alc.B., I.H.

FIGURES 35 to 39. Leg muscle, cicada nymph (*Cyclochila australasiae*).

35. Longitudinal section; fresh frozen section.
36. Cross-section; fresh frozen section.
37. Longitudinal section, relaxed fibre; note 'sarcoplasmic reticulum', and, on left, attachment of *Z*-membranes to sarcolemma (marked by asterisk). Alc.B., I.H.
38. Similar preparation; contracted fibre, showing 'striation reversal'; on left, attachment of *Z*-membrane to sarcolemma.
39. Similar preparation, showing 'fixed contractile waves'.

FIGURE 40. Leg-muscle fibre—*Neoaratus hercules* (Asilidae). Focus adjusted (on right) to pass along plane of lamella; elsewhere it intersects lamellae; note resolution of lamella into subfibrils at insertion end. Alcohol formol, I.H.

FIGURES 41 to 43. Flexor mandibulae muscle (*Periplaneta americana*).

41. Cross-section; fresh frozen section.
42. Longitudinal section, showing sarcostyles; fresh frozen section, sarcoplasm removed by refrigeration.
43. Cross-section, showing compound sarcostyles. Alc.B., I.H.

PLATE 19

FIGURE 44. Prothoracic leg-muscle fibre of grasshopper (*Caedicia olivacea*); fresh frozen section.

FIGURES 45, 46. Leg muscle of spider, *Pholcus littoralis*.

45. Cross-section; fresh frozen section.

46. Optical section along living fibre, seen through transparent leg.

FIGURES 47 to 51. Fibres of intersegmental abdominal muscle of *Thrips imaginis*, showing helicoidal striation and its development.

47. Three successive fibres, with intersegmental attachments; late nymph.

47*a*, *b*. Fragment of left-hand fibre, focused at two levels to show 'turn' of helicoid.

48. Two-striation stage, from minute nymph.

49. Three-striation stage.

50*a*, *b*. Two photographs, at very slightly different focal levels, showing transition between three- and five-striation stage, and with incipient helicoid.

51. Six-striation stage; helicoid developed.

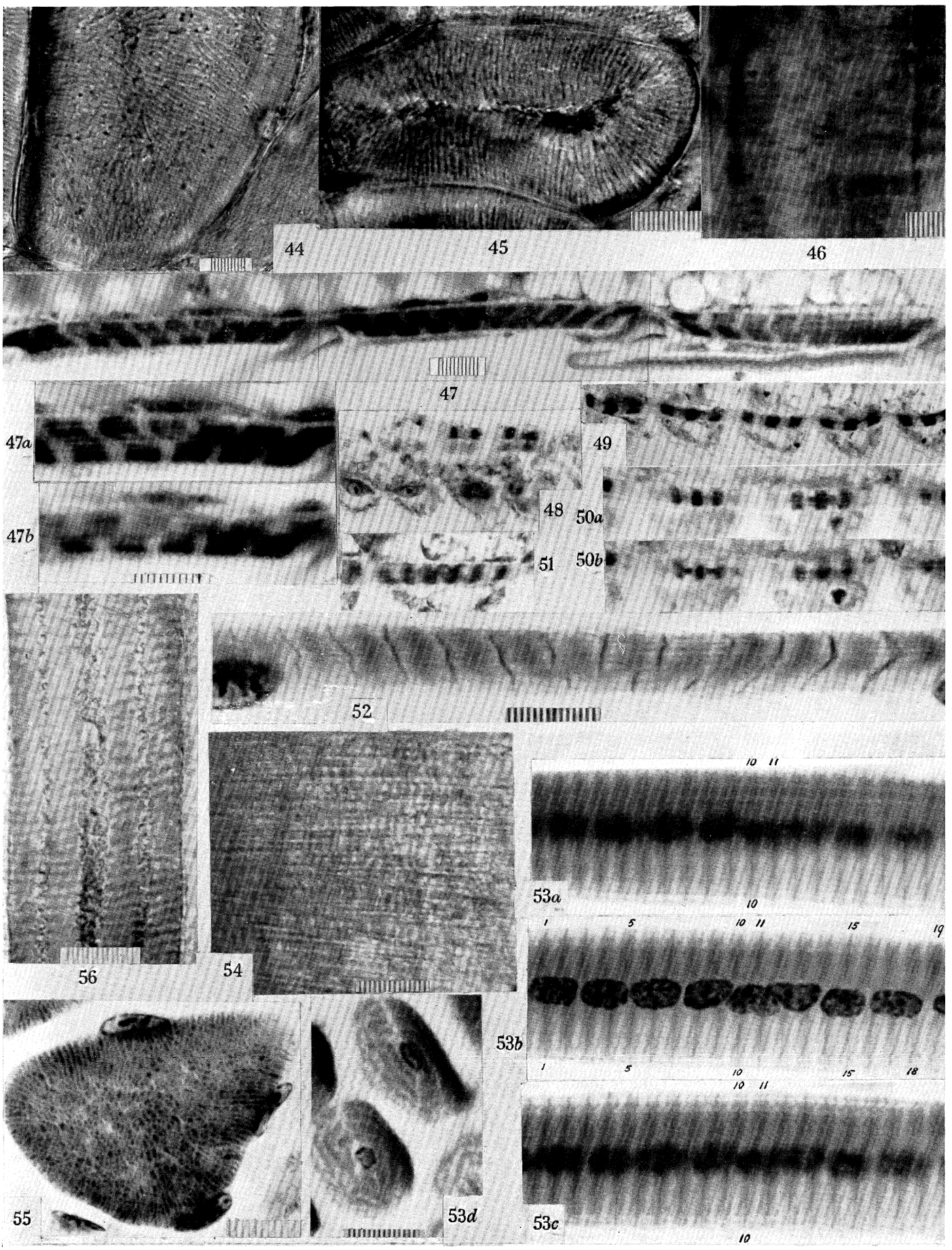
FIGURE 52. Abdominal muscle fibre, cicada nymph, showing 'vernier'.

FIGURE 53*a–d*. Leg-muscle fibre of *Tabanus imperfectus*; *a–c*, the same fibre focused at three levels (*b*, intermediate), to show helicoidal pattern of striation; *d*, cross-section.

FIGURE 54. Living fibre of *Daphnia carinata*, seen through transparent cuticle.

FIGURE 55. Leg-muscle fibre of grasshopper, *Caedicia olivacea*; cross-section showing composite character of lamellar sarcostyles around fibre margin. Alc.B., I.H.

FIGURE 56. Living muscle fibre, house-fly; optical section. Focus passes along axial column of sarcoplasm, and intersects twice the outer concentric ring of sarcoplasm. Sarcosomes visible.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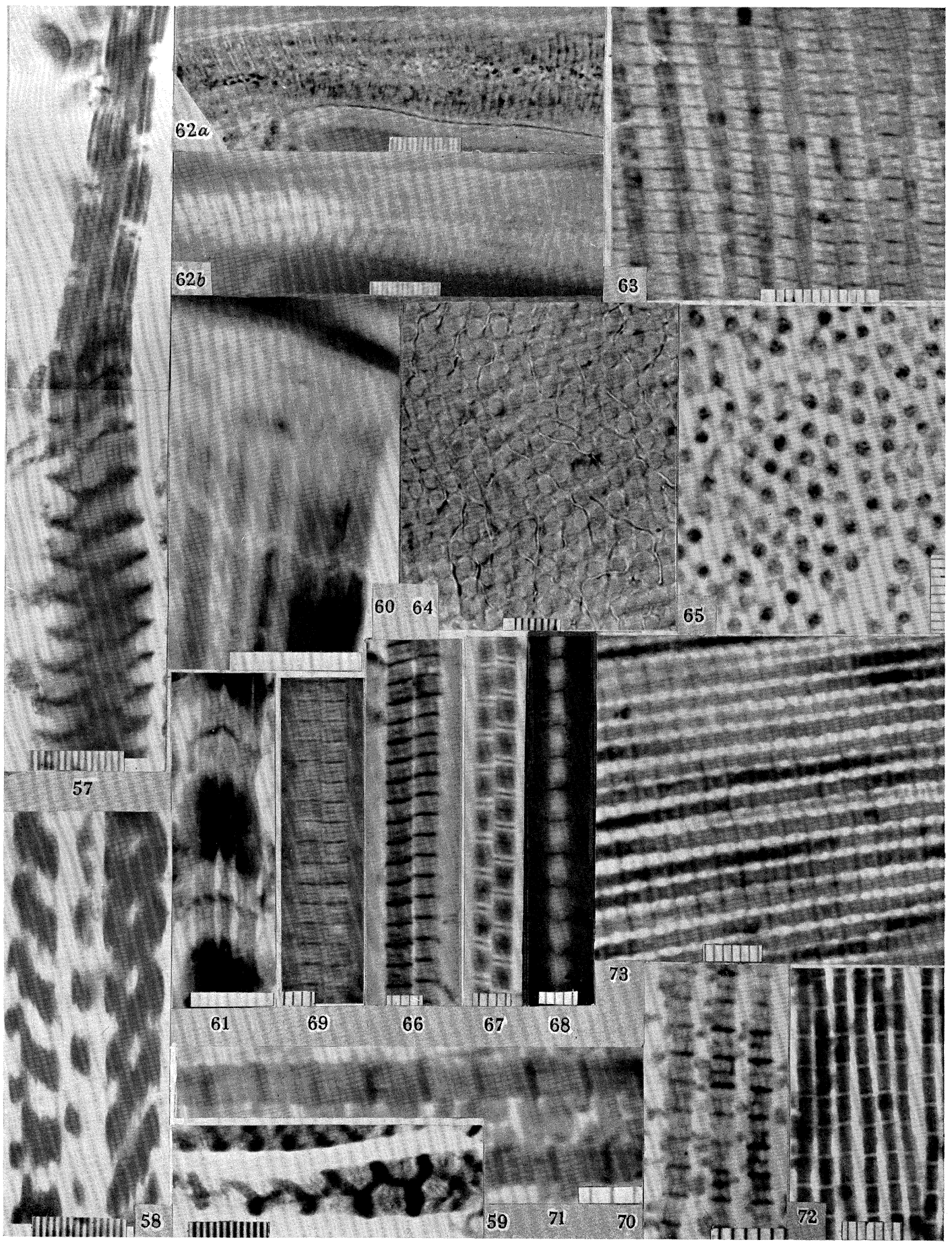
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Plate 20

Figure 57. Fibre of springing muscle of *Scolypopa australis* (Homoptera), showing 'fixed contractile wave' and helicoidal striation. A.A.T., I.H.

Figure 58. Two fibres of the same muscle; right fibre shows double helicoid; 'vernier' effect in left fibre.

Figure 59. Fragment of fibre of *Chrysopa* nymph, showing helicoidal striation.

Figure 60. Insertion end of a fibre of springing muscle of *Scolypopa australis*, photographed in ultra-violet light, with N.A. 1·95, to show composite structure of sarcostyles, and continuity of their fibrils with the tonofibrillae. A.A.T., I.H.

Figure 61. From the same muscle, to show character of *Z*-membrane; technique as in figure 60.

Figure 62*a*, *b*. Muscle fibre of house-fly, fresh, and with stationary contractile wave; *a*, with phase contrast; *b*, with polarized light and filter.

Figures 63 to 73. Wing muscle of bee (*Apis mellifica*).

63. Fragment of fresh fibre, phase contrast.
64. Cross-section of fresh frozen tissue; note air-filled tracheae.
65. Cross-section, showing subfibrillation of sarcostyles. Alc.B., I.H.
66. Fresh isolated sarcostyles; phase contrast.
67. The same, stretched; phase contrast.
68. Stretched fresh sarcostyle; polarized light and filter.
69. Two fresh isolated sarcostyles; phase contrast, polarized light and filter.
70. Three sarcostyles, prepared to show component myofibrils. Alc.B., I.H.
71. Sarcostyles, showing myofibrils, but with unstained *M*-membrane. A.A.T., I.H., ultra-violet light.
72. Group of sarcostyles, prepared by routine Bouin fixation, shrunken and without resolution of component fibrils.
73. Fragment of longitudinal section, showing *Z*-membrane. Carnoy, I.H.

Plate 21

Figure 74. *Apis mellifica*; wing muscle. Note sarcolemma bordering right margin of fibre (asterisk), with attachments at *Z*-membrane.

Figures 75 to 81. Wing muscle of wild bee, *Halictus speculiferus*.

75. Cross-section, showing subfibrillation of sarcostyles. Alc.B., I.H.
76. Sarcostyles, slightly stretched, showing component myofibrils. Alc.B., I.H.
77. The same, unstretched. Alc.B., I.H.
78. Fragment of a section along a fibre that has been placed for 1 h in egg-white before fixation; note *N*-granules. Alc.B., I.H.
79. Similar section, 2 h immersion before fixation; shortened sarcostyles, with 'striation reversal'. Alc.B., I.H.
80. Sarcostyles with unstained *M*-membrane. AlcB., I.H.
81. Sarcostyle, with pale band adjacent to *M*-membrane. Alc.B., I.H.

Figure 82. Sarcostyles from wing-muscle of *Sericesthis pruinosa* (Coleoptera). A.A.T., I.H.

Figures 83, 84. Sarcostyles from wing muscle of *Eurymela distincta* (Homoptera, Jassidae).

83. Fresh sarcostyle, relaxed. Phase contrast.
84. The same, shortened. Phase contrast, polarized light and filter.

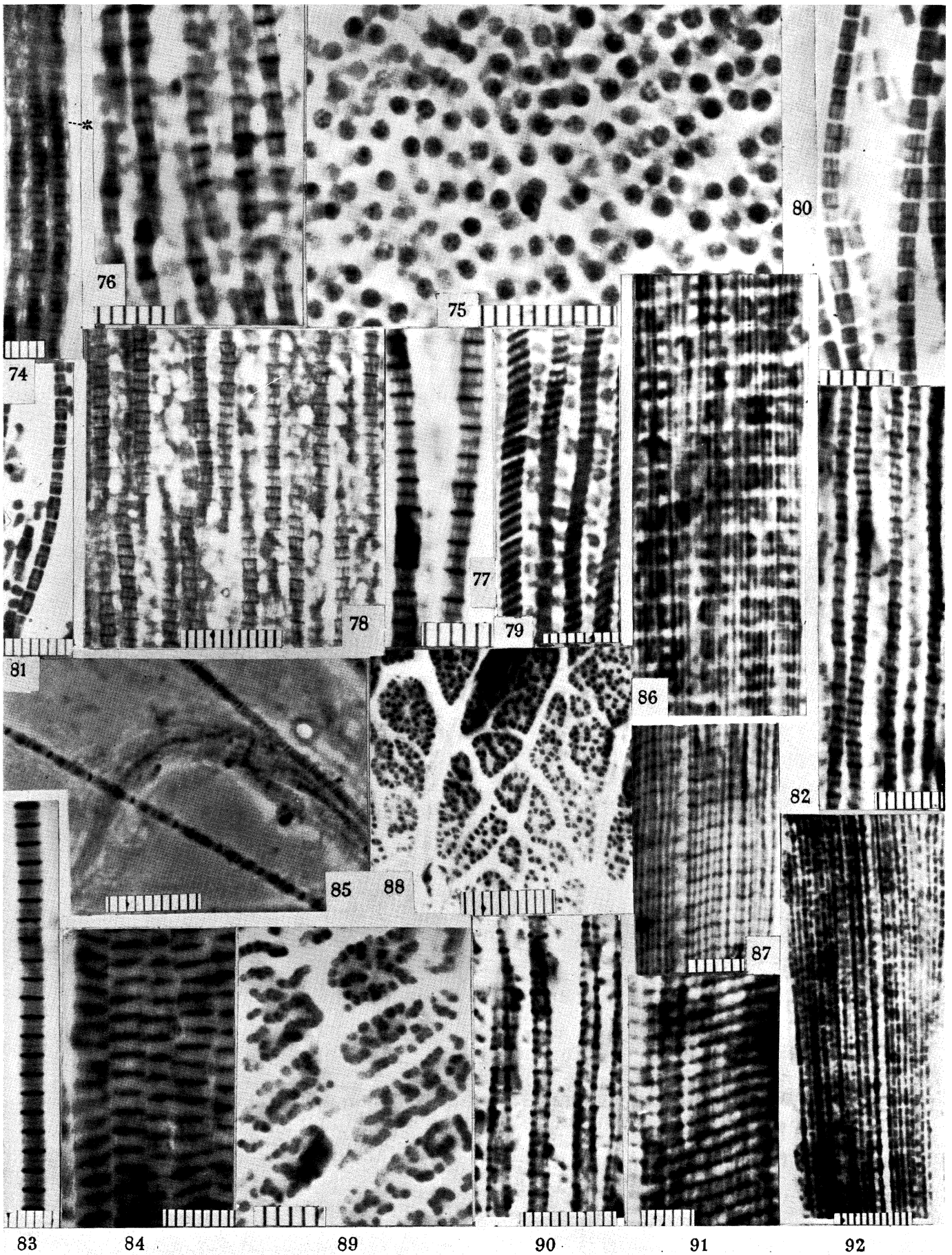
Figures 85 to 88. Wing muscle of *Siphanta acuta* (Homoptera, Flatidae).

85. Fresh isolated sarcostyles, stretched and unstretched; phase contrast.
86. Relaxed fibre, longitudinal section; *Zf* visible, but not *Mf*; sarcosomes present. A.A.T., I.H.
87. Similar section; *Q*-band unstained, *Z*- and *M*-membranes visible. Alcohol formol trichloracetic acid, I.H.
88. Cross-section; in places there is a just perceptible subfibrillation. Alc.B., I.H.

Figures 89 to 91. Wing-muscle of *Cyclochila australasiae* (Homoptera, Cicadidae).

89. Fragment of cross-section, showing composite structure of sarcostyles. Alcohol formol trichloracetic, I.H.
90. Group of sarcostyles, showing component myofibrils. A.A.T., I.H.
91. Contracted sarcostyles. A.A.T., I.H.

Figure 92. Wing-muscle fibre of *Erythroneura ix* (Jassidae); the section is from the metathoracic tergo-sternal muscle, which has unusually thin sarcostyles. Alc.B., I.H.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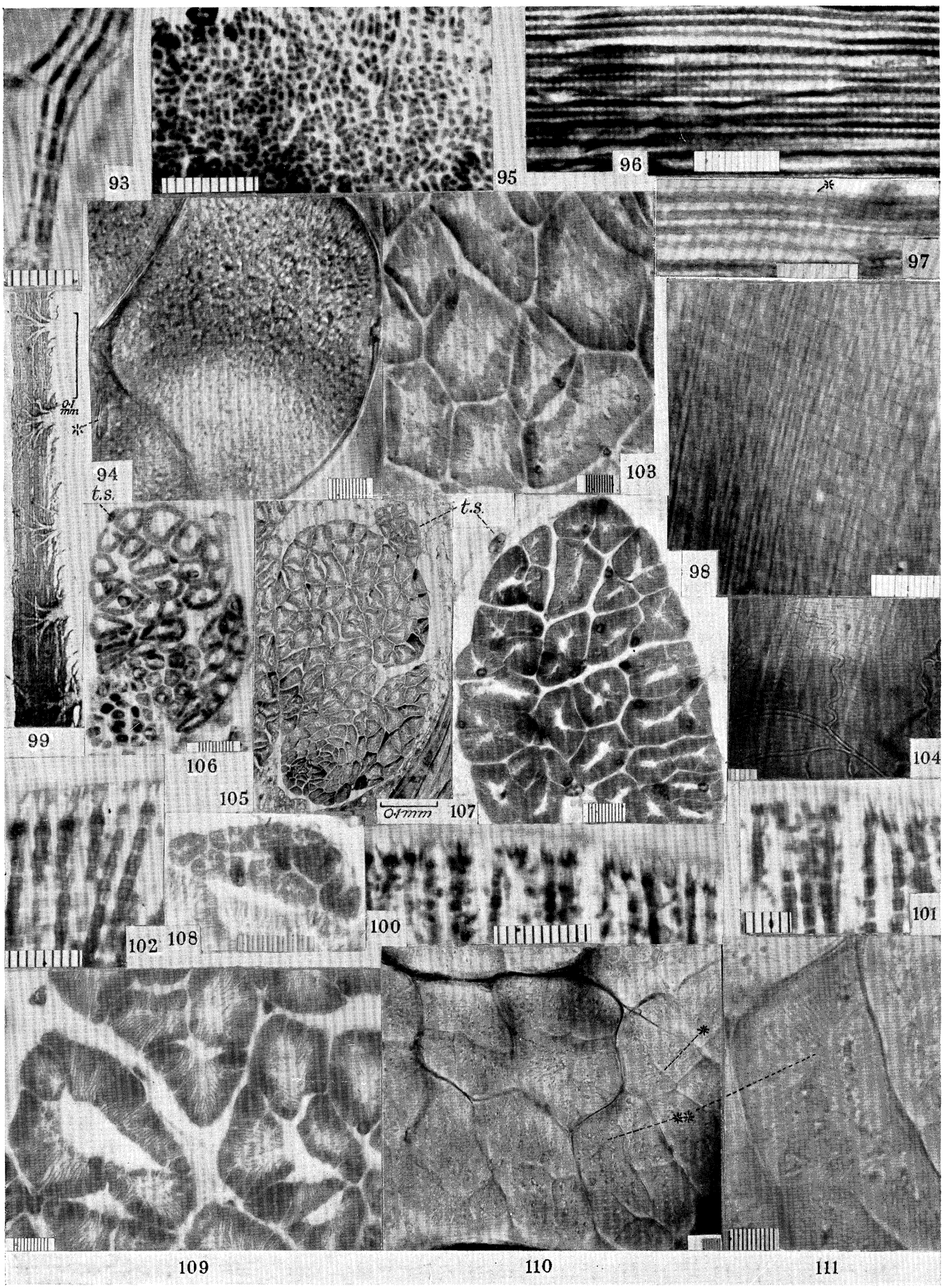
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Plate 22

Figures 93 to 95. Wing muscle of *Caedicia olivacea* (Orthoptera, Tettigoniidae).

93. Fresh sarcostyles; phase contrast.
94. Fresh fibre, showing Cohnheim pattern and sarcosomes; frozen section.
95. Cross-section. Alc.B., I.H.

Figures 96, 97. Wing muscle, *Scolia bimaculata* (Hymenoptera).

96. Showing complete *Z*- and *M*-membranes. Alc.B., I.H.
97. The same, showing their attachment to sarcolemma (asterisk).

Figure 98. Wing muscle of *Heteronympha merope* (Lepidoptera); fresh frozen section, showing continuous *Z*-membrane. Phase contrast, polarized light and filter.

Figure 99. Fragment of wing muscle of *Sisyromyia aurata* (Bombyliidae); the section grazes along the surface of the fibre, and shows tracheae within the sarcolemma. Da Fano process.

Figures 100, 101. Wing muscle of *Scolia bimaculata* (Hymenoptera); the section is at the muscle attachment, and shows connexion of the myofibrils of the sarcostyles with the tonofibrillae. Alc.B., I.H.

Figure 102. The same, from *Paracolletes* sp. Alc.B., I.H.

Figures 103 to 108. Wing muscle of *Blattella germanica.*

103. Cross-section of a group of typical fibres of wing musculature. Carnoy, I.H.
104. Fragment of fresh fibre, showing *Z*-membrane.
105. Cross-section of first tergo-coxal muscle of mesothorax, with attached small tergo-sternal muscle (*t.s*). Adult.
106. The same, from a 3 mm nymph.
107. Part of same, from a 6 mm nymph, showing fibre cleavage. Carnoy, I.H.
108. Developing tergo-sternal muscle from late nymph. Carnoy, I.H.

Figures 109 to 111. Fibre cleavage in *Periplaneta americana.*

109. Fragment of section through mesothoracic tergo-coxal muscle. Alc.B., I.H.
110. The same; frozen section of fresh tissue.
111. Fragment from figure 110, at higher magnification.

Plate 23

Figure 112. Fibre from wing musculature, *Periplaneta americana*. Alc.B., I.H.

Figures 113, 114. *Gryllotalpa australis*, wing muscle.

113. Piece of coxal remotor muscle, fresh in glycerine to show anastomosing air-filled tracheae within muscle.

114. Nymphal muscle, showing fibre cleavage. Alc.B., I.H.

Figures 115, 116. *Orthodera ministralis*, wing muscle.

115. Adult fibre. Alc.B., I.H.

116. Nymphal muscle, showing fibre cleavage. Alc.B., I.H.

Figures 117 to 124. *Acridopeza reticulata*.

117. Cross-section of mesothoracic leg muscle, female; frozen section.

118. The same, from male.

119. Cross-section of mesothoracic first tergo-coxal muscle, from 4 mm male nymph. Alc.B., I.H.; *tr.*, tracheae.

120. Fragment of same, from 1 cm male nymph, showing beginning of fibre cleavage. Alc.B., I.H.

121. Fragment of same, from 15 mm nymph, cleavage advanced. Alc.B., I.H.

122. Cross-section of entire pleuro-tergal muscle of adult male.

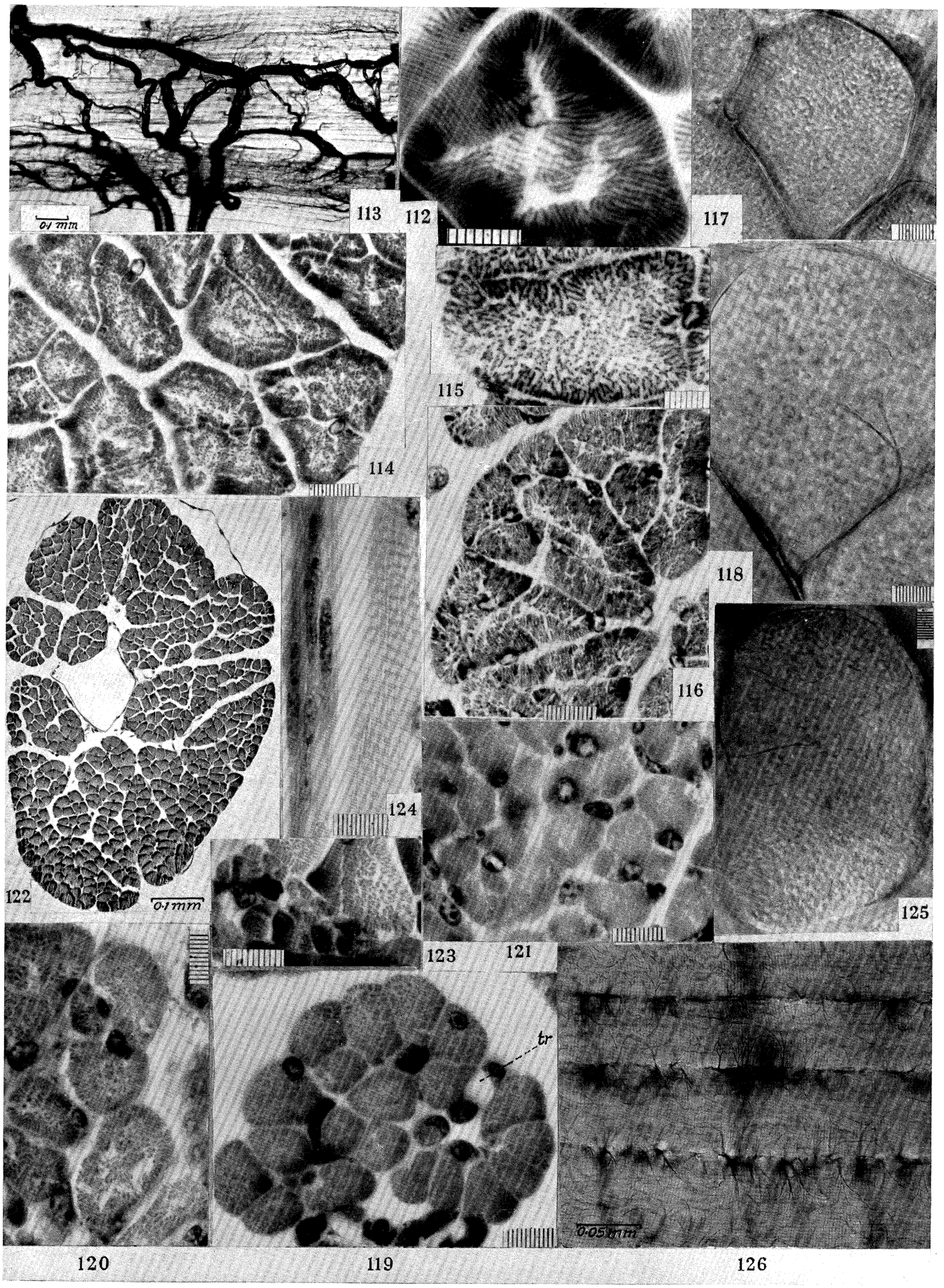
123. The same, from a 4 mm male nymph; the large fibre alongside the group of small immature fibres is a functional leg-muscle fibre. Alc.B., I.H.

124. Longitudinal section of the same; 4 mm nymph. Alc.B., I.H.

Figures 125, 126. *Chortoicetes terminifera*.

125. Fresh fibre of wing musculature; frozen section.

126. Group of fresh fibres in glycerine, showing air-filled tracheae.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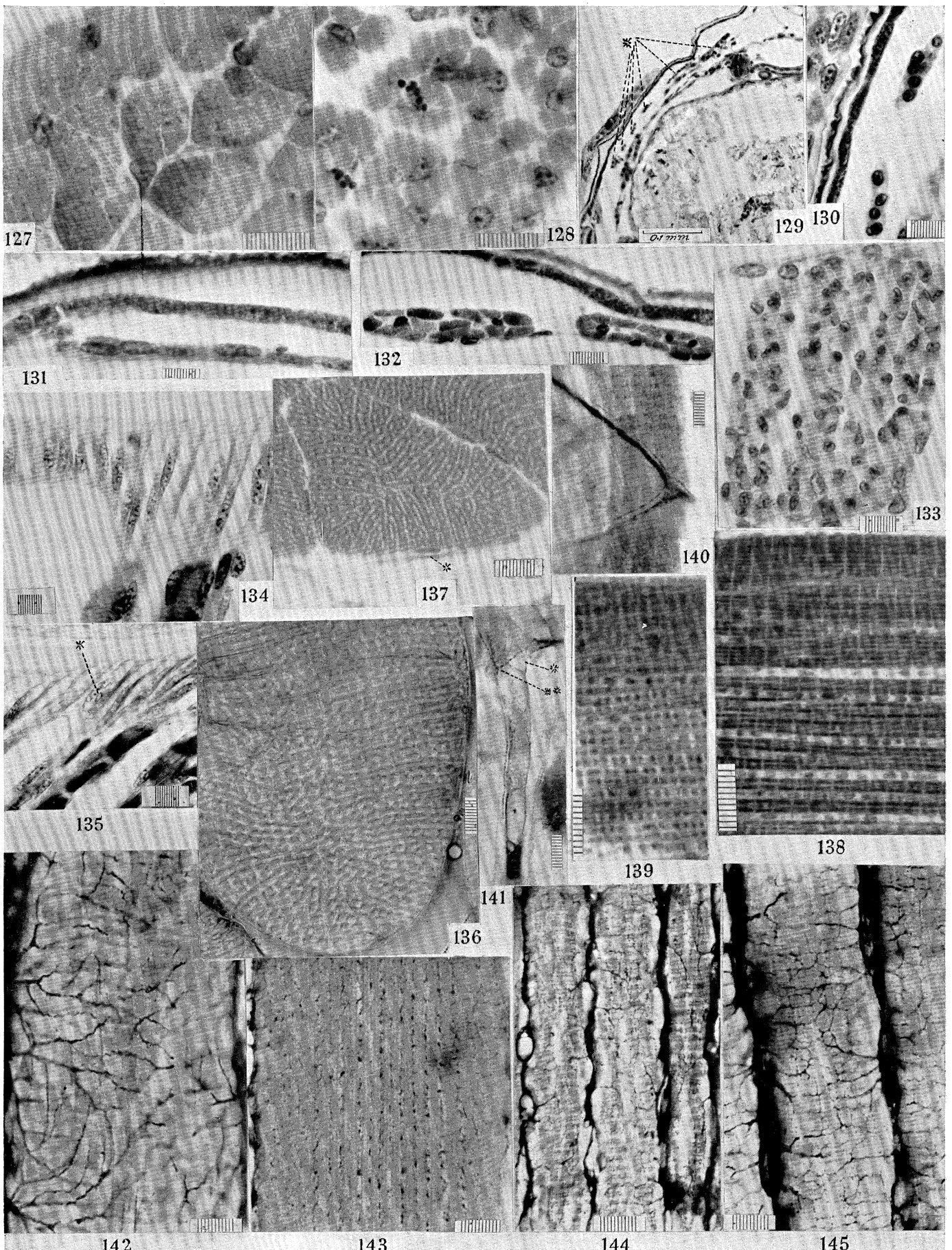
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Plate 24

Figures 127 to 135. *Chortoicetes terminifera.*

127. Fragment of subalar muscle, from young nymph, showing beginning of fibre cleavage. Carnoy, I.H.
128. Similar fragment, from advanced nymph, near end of cleavage phase. Carnoy, I.H.
129. Cross-section of left upper quarter of mesothorax of first instar nymph; the asterisk indicates the six rudimentary fibres from which the massive dorsal longitudinal wing muscle will develop.
130. Part of same section, at higher magnification, showing two of the rudimentary fibres. Carnoy, I.H.
131. Longitudinal section of one of the rudimentary fibres; on left is seen its attachment to overlying epidermis, above which is the chitinous covering. Carnoy, I.H.
132. Cross-section, similar to figure 130, showing beginning of fibre cleavage. Carnoy, I.H.
133. Similar cross-section from late nymph. The photograph represents one of the six fibre bundles of the dorsal longitudinal muscle, product of repeated cleavage of an original rudimentary nymphal fibre. Carnoy, I.H.
134. Section along outer margin of muscle attachment, from final instar nymph, showing recruitment of new epidermal cells into zone of attachment. Carnoy, I.H.
135. Similar section, from middle of muscle attachment, showing incipient mitosis in cell with tonofibrillae. Carnoy, I.H.

Figures 136 to 145. *Cyclochila australasiae*; wing muscle.

136. Adult muscle fibre, fresh frozen section; note air-filled tracheae.
137. The same, with motor-nerve ending (asterisk). Carnoy, Willis's silver process.
138. Longitudinal section; sarcosomes visible. Alc.B., I.H.
139. Similar section, showing sarcosomes. Bouin fixation, I.H.
140. Fragment of intramuscular nerve from wing musculature, showing bifurcation of the large fibre, and of accompanying delicate fibre. Carnoy, Willis's silver process.
141. Similar but thicker fragment; massive nerve fibre (one asterisk) and delicate fibre (two asterisks) branch simultaneously at point indicated by asterisks. Carnoy, Willis's silver process.
142, 143. Longitudinal sections of adult fibres, with impregnated tracheae. In figure 143 the section transects most of the lamellae, between which the tracheae lie; in figure 142 it is in the plane of the interlamellar space, and therefore shows the full expanse of the branching tracheae. Da Fano preparation.
144. Developing tracheae; the section shows three young muscle fibres, from a late nymph, into which the tracheae are growing. Da Fano preparation.
145. Later stage of same, showing development of closed tracheal net. Da Fano preparation.

Plate 25

Figures 146 to 165. *Cyclochila australasiae*, wing muscle.

146. Cross-section of a developing fibre bundle from late nymph, showing penetration of fibres by tracheae. Da Fano preparation.

147, 148. Cross-membranes in adult fibre. In figure 147 the section seems to be along a lamella; in figure 148 it plainly transects the lamellae, except in the left third of the section (for orientation see figure 138). Both figures show the *Z*-membrane, and particularly the delicate *M*-membrane, completely transecting the interlamellar spaces. Carnoy, Willis's silver process.

149. Fragment showing *Z*-membrane. Alcohol, trichloracetic acid, I.H.

150. Motor-nerve ending; gold chloride.

151. Initial stage in development of tergo-sternal muscle, showing fusion of myoblasts into a column; from a 3-day nymph. Carnoy, I.H.

152. Slightly later stage of same; fibrillation beginning, from 3-day nymph. Carnoy, I.H.

153. Cross-section of rudiment of median dorsal longitudinal muscle, from a 1 cm nymph (drawn, in position, in figure 9*a*). Carnoy, I.H.

154. Longitudinal section of same, from opposite half of same nymph.

155. Cleavage of same into five parts. Alc.B., I.H.

156. Fragment of the section shown in figure 9*b*, i.e. early in second cleavage phase. Alc.B., I.H.

157. Fragment of section shown in figure 9*c*, i.e. at end of second cleavage phase. Alc.B., I.H.

158. A single fibre from same nymph; longitudinal section.

159. Cross-section of single fibre at beginning of third cleavage phase. Alc.B., I.H.

160. Slightly later stage in development of same, showing fibril increase. Alc.B., I.H.

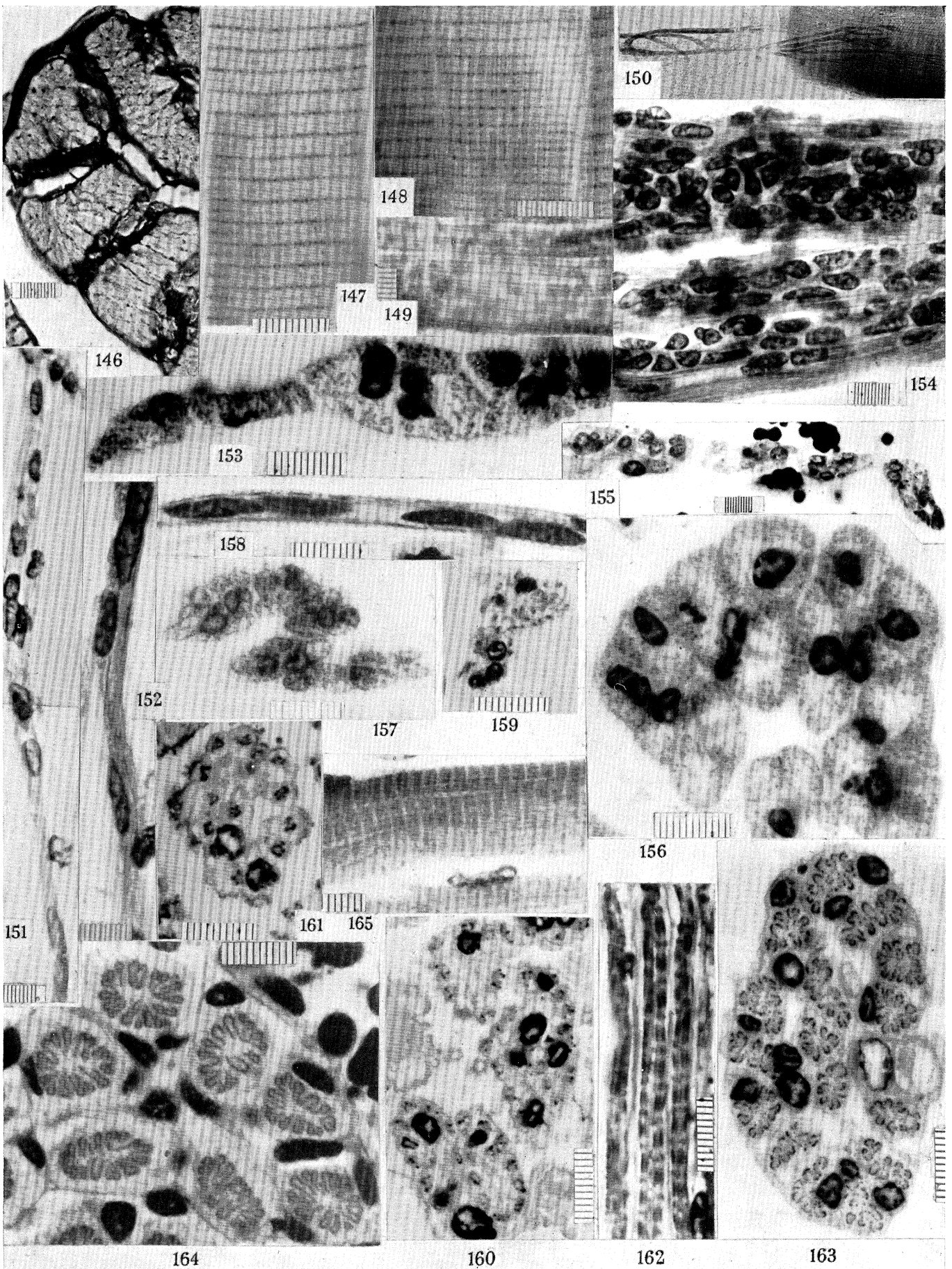
161. The same, showing separation of fibres in a developing fibre bundle. Alc.B., I.H.

162. Longitudinal section of same.

163. Later stage in development of fibre bundle, showing 'rosette' pattern. Alc.B., I.H.

164. Later stage of same. Alc.B., I.H.

165. Longitudinal section of fairly advanced fibre, showing 'vernier'. Alc.B., I.H.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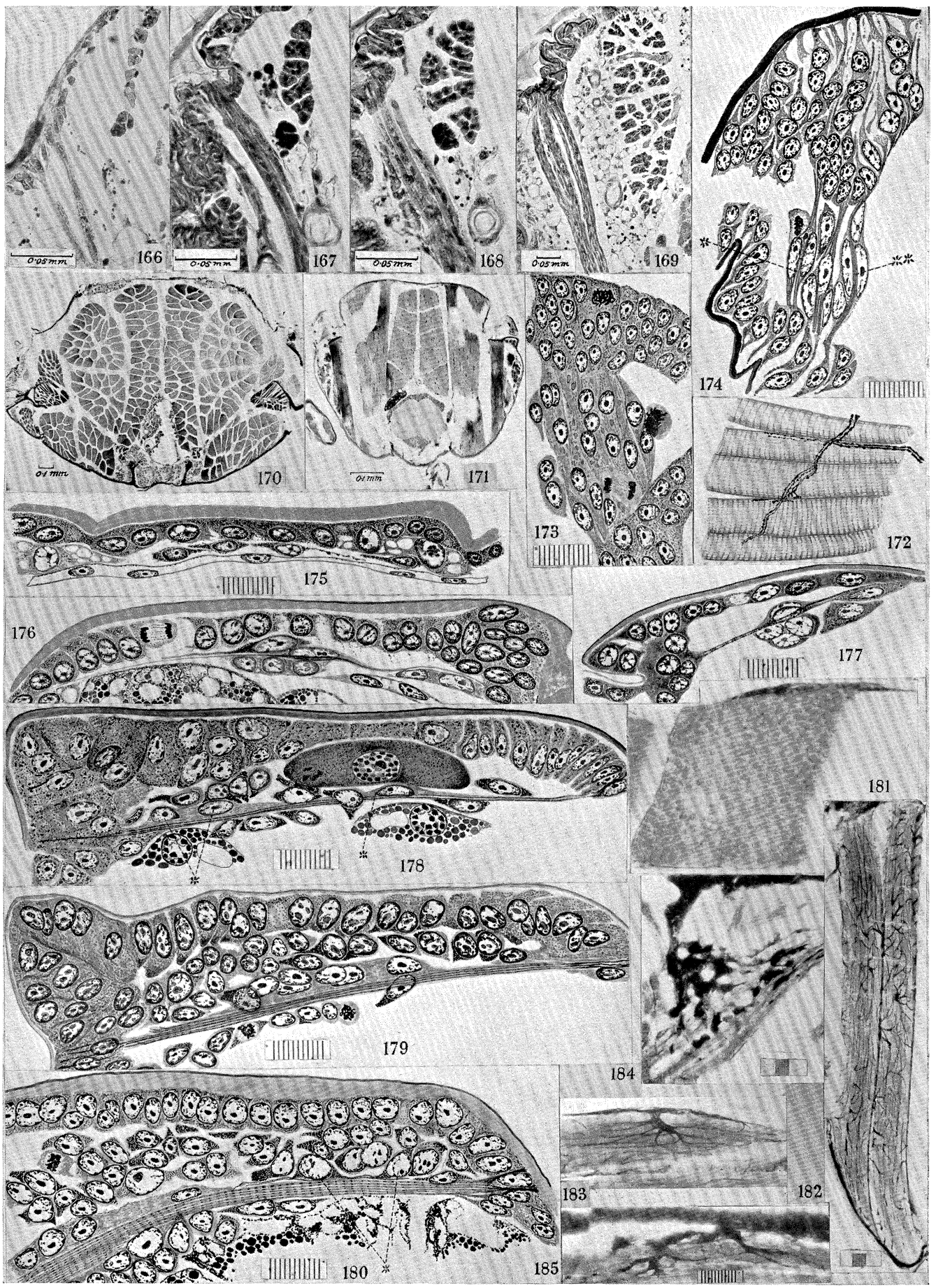
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Plate 26

Figures 166 to 169. *Pauropsalta encaustica*. Cross-sections of left upper part of mesothorax of four nymphs, showing progressive development of the dorsal longitudinal muscle (transected on right) and of tergo-sternal muscle (longitudinally cut on left). Figures 166 to 168 from 6 mm nymph; figure 169 from 1 cm nymph. Alc.B., I.H.

Figure 170. *Anipo brunneus* (Jassidae); cross-section of mesothorax, adult.

Figures 171 to 185. *Erythroneura ix* (Jassidae).

171. Cross-section of mesothorax; adult.
172. Fragment of tergo-pleural muscle (wing adjustor), showing innervation. Da Fano process.
173. Early phase in development of tergo-pleural muscle. Alc.B., I.H.
174. The same, later phase.
175. Section along roof of mesothorax of a 0·7 mm nymph, showing, above the trachea, the four myoblasts from which the dorsal longitudinal muscle will develop. Alc.B., I.H.
176. Similar section, from a 0·9 mm nymph, showing pioneer myoblast. Alc.B., I.H.
177. Two-fibril stage (from oblique tergal muscle).
178. Three-fibril stage (dorsal longitudinal muscle), with three muscle nuclei (indicated by asterisk). Alc.B., I.H.
179. Seven-fibril stage. Alc.B., I.H.
180. About forty-fibril stage. Two myoblasts (marked by asterisk) are in process of incorporation into growing fibre. Alc.B., I.H.
181. Cross-section of a fibre of the dorsal longitudinal muscle, showing motor-nerve ending. Da Fano preparation.
182. Section along the two fibres that comprise the tergo-sternal (left) and tergo-coxal (right) wing muscles, showing tracheae. Da Fano preparation.
183. Tracheal end-cell, in wing-muscle fibre. Da Fano preparation.
184, 185. Two stages in development of tracheae. Figure 183 represents a section along the oblique tergal muscle, and shows invasion by tracheal cells; figure 184 shows penetration of a muscle fibre by two tracheal cells. Da Fano preparation.

Plate 27

Figures 186 to 193. *Erythroneura ix* (continued).

186. Cross-section of mesothorax of young nymph, showing rudiment of dorsal longitudinal muscle (asterisk).
187. Cross-section of muscle rudiment of same nymph (opposite side). A.A.T., I.H.
188. The same, from a more advanced nymph. Alc.B., I.H.
189. Fragment of fibre of dorsal longitudinal muscle at three-fibril stage. Carnoy, I.H.
190. Section along fibre of dorsal longitudinal muscle, from half-grown nymph, showing sarcolemma (asterisk), in continuity with basement membrane of epidermis. Alc.B., I.H.
191. Cross-section of wing-muscle fibre from late nymph, showing cleavage into fibril columns, and nuclear invasion. Alc.B., I.H.
192. Fragment of wing-muscle fibre, showing split sarcostyle. Alc.B., I.H.
193. Motor-nerve ending on wing-muscle fibre; note entrance of two separate nerve-fibres into end-organ. Da Fano preparation.

Figures 194, 195. *Eurinoscopus viridis* (Jassidae).

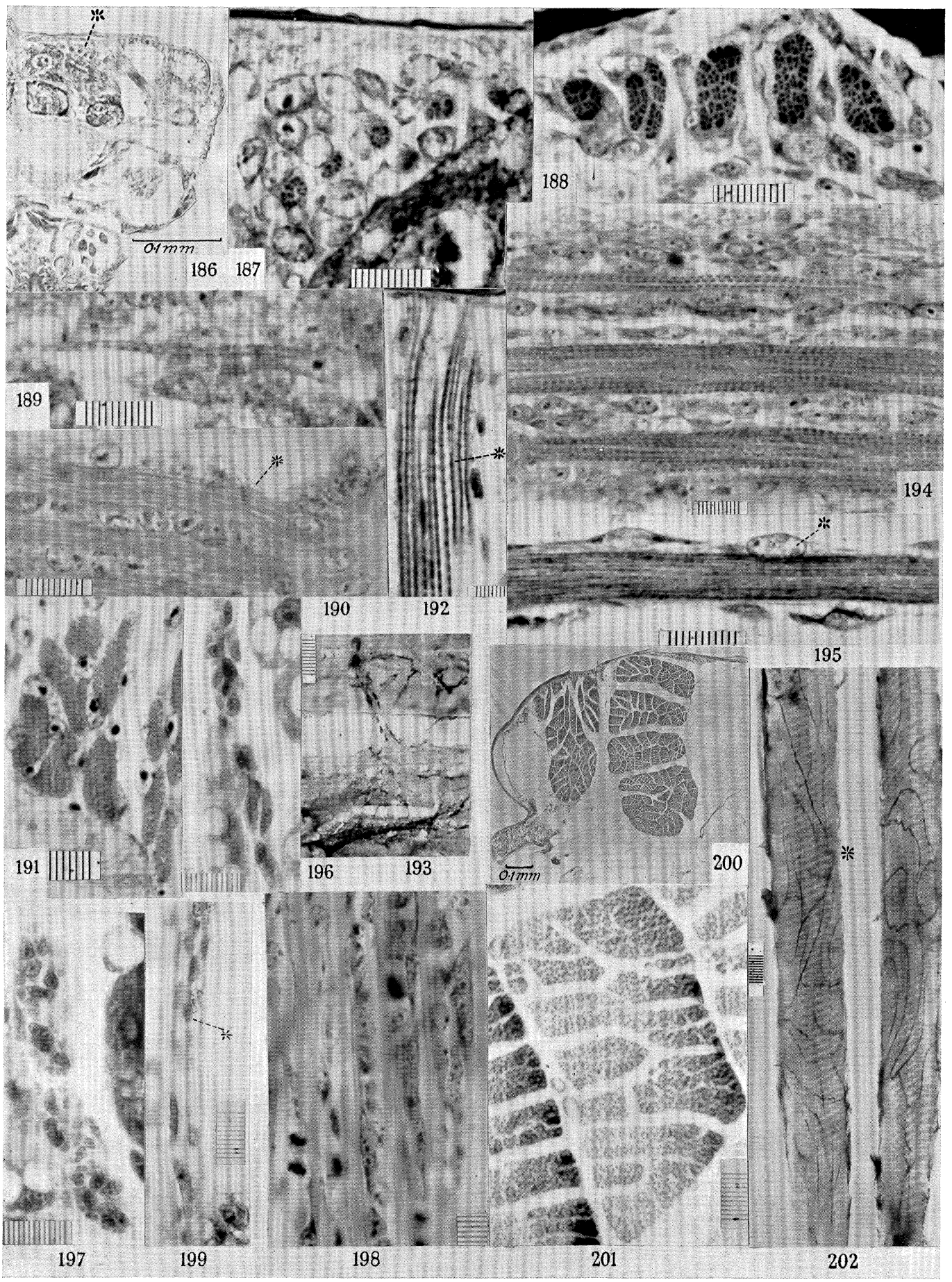
194. Section along developing wing muscle, showing free myoblasts. Alc.B., I.H.
195. Fragment from a rather earlier stage, showing, above the fibre, two myoblasts, of which one (asterisk) has elongated the full length of the photograph, and is in process of incorporation into fibre. A.A.T., I.H.; phase contrast.

Figures 196 to 199. *Anipo brunneus* (Jassidae).

196. Cross-section of developing dorsal longitudinal muscle, showing the five rudimentary fibres in process of cleavage. Alc.B., I.H.
197. Similar section, rather later stage. Alc.B., I.H.
198. Longitudinal section from nymph after completion of fibre cleavage, showing free myoblasts between growing fibres. Alc.B., I.H.
199. Section along fragment of same, showing myoblast filament extending along a young fibre; cell of origin indicated by asterisk.

Figures 200 to 202. *Scolypopa australis* (Ricaniidae).

200. Left half of adult mesothorax in cross-section, showing transected dorsal longitudinal muscle, and to left of this, part of tergo-sternal muscle. Alc.B., I.H.
201. Cross-section of single fibre of longitudinal muscle. Alc.B., I.H.
202. Section along two fibres of wing muscle, showing intracellular tracheae. Da Fano process.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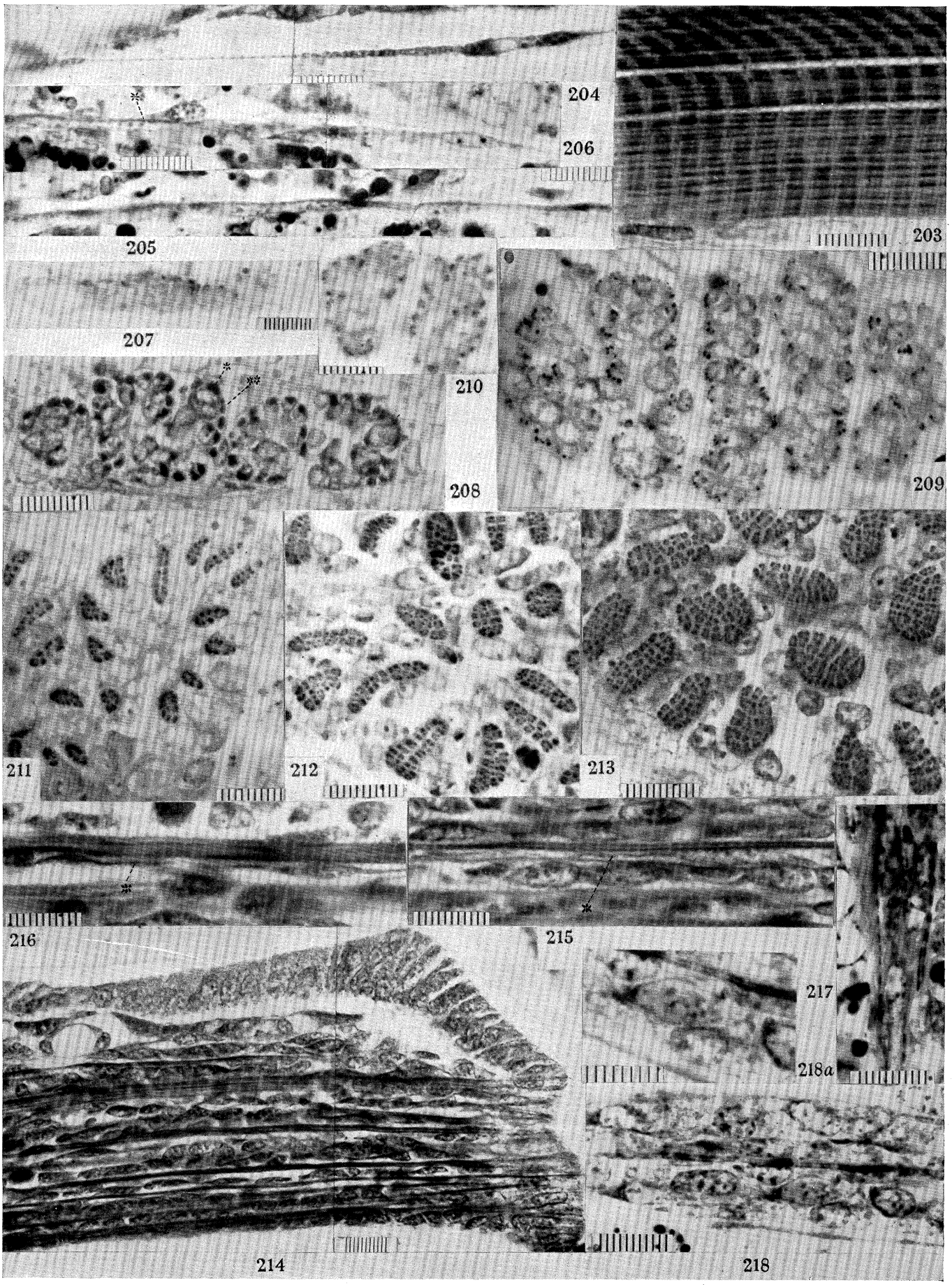
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Plate 28

Figures 203 to 218. *Scolypopa australis* (continued).

203. Adult wing-muscle fibre, longitudinal section. Carnoy, I.H.
204. Section along roof (hind half) of mesothorax of 1 mm nymph, showing pioneer myoblasts of dorsal longitudinal muscle. Alc.B., I.H.
205. Section along an initial muscle-fibre rudiment, from a rather larger nymph (dorsal longitudinal muscle). Alc.B., I.H.
206. Adjacent section from same, showing free myoblasts, and a fragment of fibre with cross-striation.
207. Cross-section from rather later nymph, showing incipient cleavage; the minute black dots are transected fibrils. Trachea to left. Alc.B., I.H.
208. Similar section, but from an older nymph, showing cleavage of muscle rudiment into five columns (fragment of trachea to right). Alc.B., I.H.
209. Later stage of same, fibre cleavage completed. A.A.T., I.H.
210. Fragment from a slightly earlier stage, showing single fibril stage in development of future wing muscle fibre. A.A.T., I.H.
211. Cross-section of part of muscle rudiment, showing definitive fibres considerably more advanced than in figure 209.
212. Similar section, later stage. A.A.T., I.H.
213. Similar section, still later stage. A.A.T., I.H.
214. Section along anterior third of developing dorsal longitudinal wing muscle, from young nymph, showing free myoblasts among developing fibres. A.A.T., I.H.
215. Fragment of same, to show filamentous outgrowth from myoblast.
216. Another fragment, showing end-to-end fusion of myoblasts; myoblast to left not quite focused.
217. Fragment of longitudinal section of stage shown in figure 210; note one- and two-fibril stages, with cross-striation. A.A.T., I.H.
218. Similar section, showing two-fibril stage. A.A.T., I.H.

218*a*. Fragment of figure 218, enlarged to show *Z*-membrane.

Plate 29

Figures 219 to 222. *Scolypopa australis* (continued).

219. Section along developing dorsal longitudinal muscle (anterior half) at a stage rather earlier than that of figure 214, most of the fibres being at the one- to four-fibril stage (cf. figure 209); note intense mitotic activity among myoblasts. A.A.T., I.H.

220, 220*a*. Fragment showing incorporation of formerly free myoblast into a young muscle fibre; in order to bring out the full length of the myoblast the drawing (figure 220*a*) has been made, making full use of focus adjustment.

221. Fragment of a longitudinally cut developing fibre, showing helicoidal striation; note free myoblasts to side of fibre. (From same nymph as figure 212.)

222. Cross-section of muscle rudiment at about stage shown in figure 209; poor resolution of fibrils, but good definition of sarcolemma. Alc.B., I.H.

Figures 223 to 226. *Perkinsiella saccharicida* (Delphacidae).

223. Section along roof of thorax of minute nymph, showing a single pioneer myoblast. A.A.T., I.H.

224. Cross-section of muscle rudiment, from a minute nymph, showing initial cleavage into five parts. The five rudimentary fibres are indicated by asterisk; below are some free myoblasts. Trachea below muscle rudiment. A.A.T., I.H.

225. Section along one of the five developing fibre columns, from a rather later nymph, showing, marginally, the young fibres, enclosing a core of crowded myoblasts. A.A.T., I.H.

226. Cross-section of developing dorsal longitudinal muscle of half-grown nymph, showing early stage of fibre enlargement. A.A.T., I.H.

Figures 227 to 233. *Bathylus albicinctus* (Cercopidae).

227. Cross-section of mesothorax of minute nymph, taken just median to left wing base (not included). The six parent fibres of the future dorsal longitudinal wing muscle are indicated by asterisk. *tr.* trachea. A.A.T., I.H.

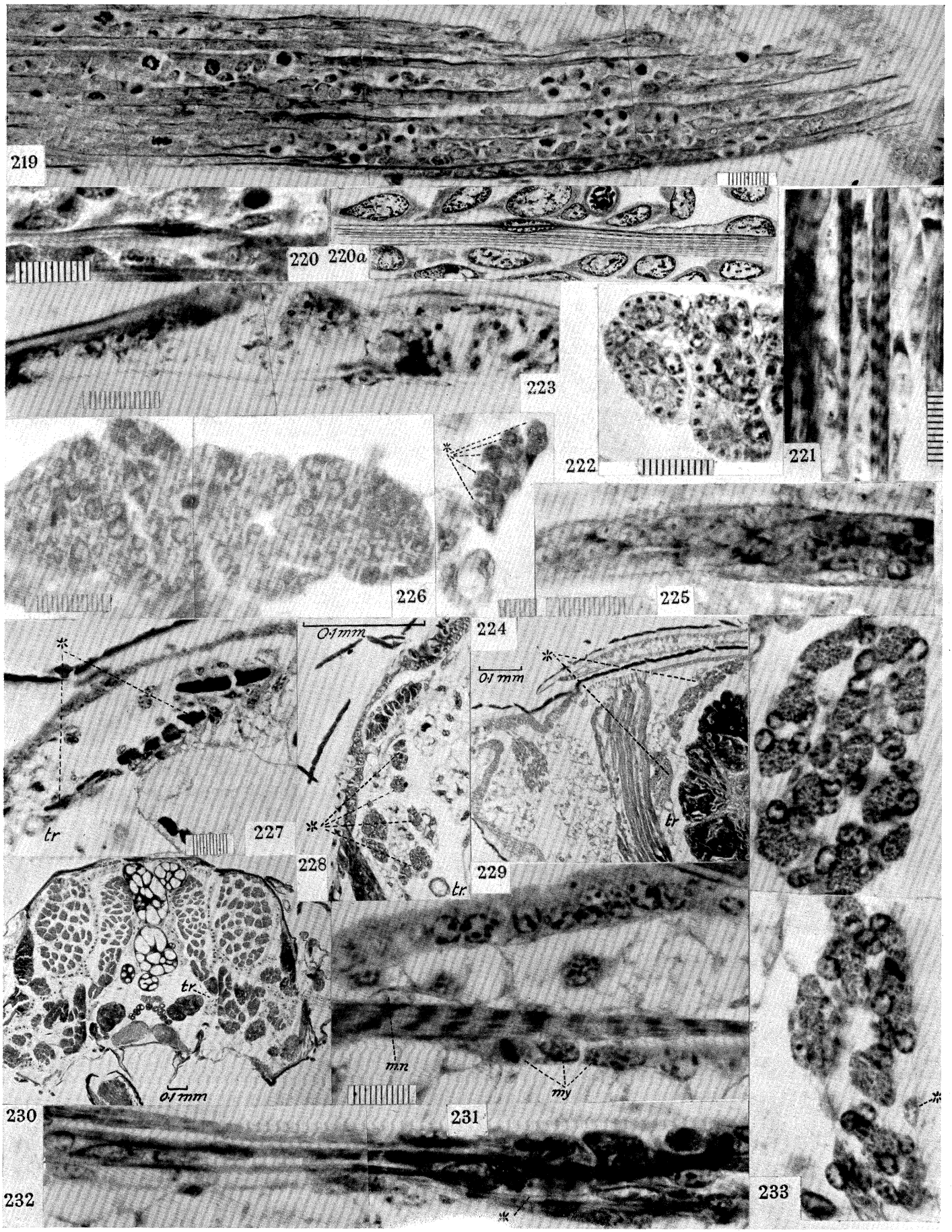
228, 229. Similar section from two older nymphs, showing growth of wing musculature. *tr.* trachea. A.A.T., I.H.

230. Cross-section of mesothorax, adult female. *tr.* trachea.

231. Section along roof of mesothorax of young nymph, showing one of the six nymphal fibres cut longitudinally; note distinction between muscle nuclei (*m.n*) and myoblasts (*my*).

232. Section along developing wing muscle from a young nymph. The section, which represents about half the length of the muscle, shows a bundle of four daughter fibres, progeny of a single nymphal fibre. On extreme left are three muscle nuclei and two myoblasts. In right half of photograph are nuclei and myoblasts (one in mitosis), but mostly too crowded for observation. From one of the myoblasts (asterisk) a filamentous outgrowth has formed. A.A.T., I.H.

233. Part of cross-section, shown at low magnification in figure 229; the section shows the progeny of the third and fourth nymphal fibres, cleavage about two-thirds complete.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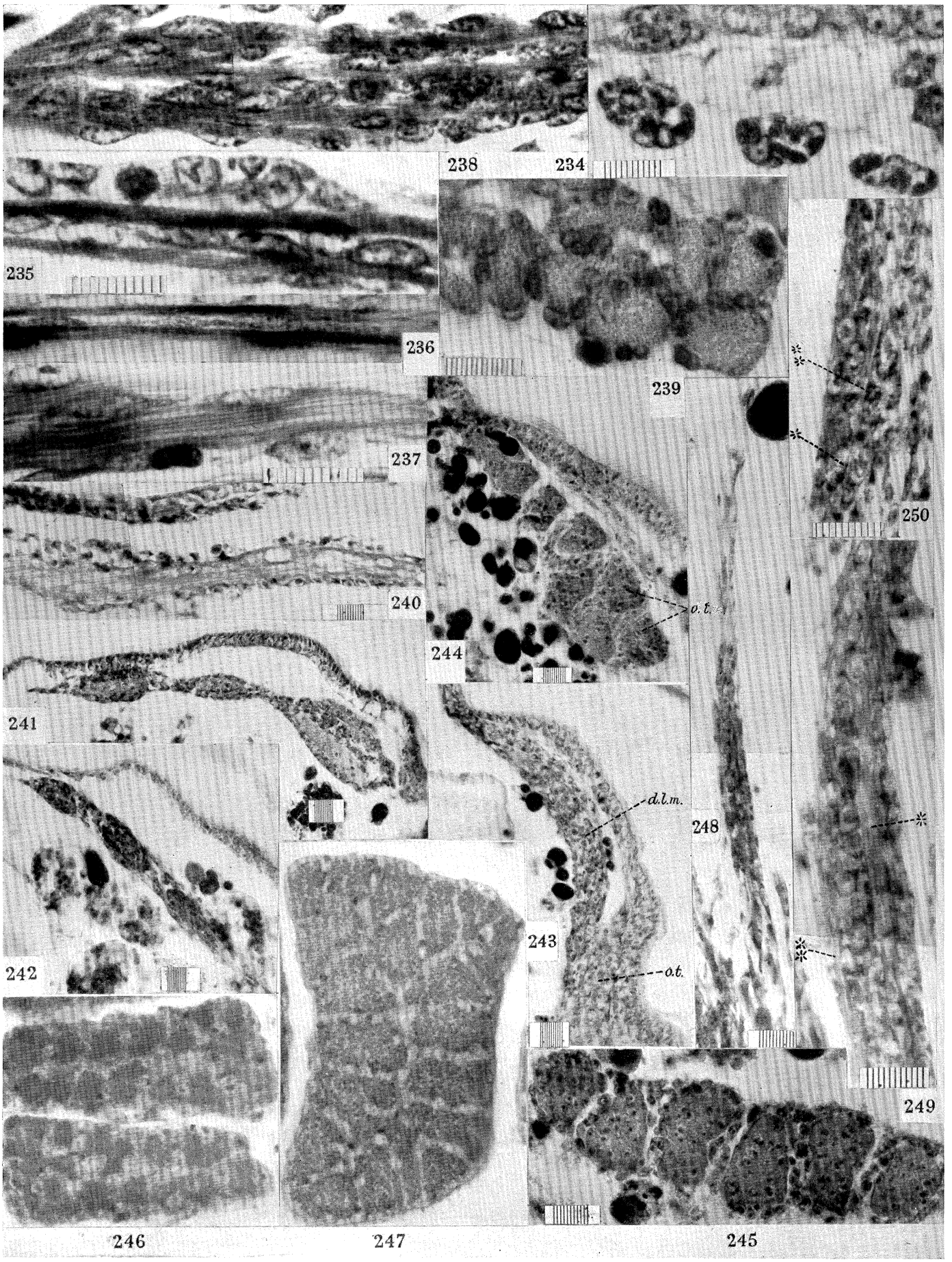
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Plate 30

Figures 234 to 239. *Bathylus albicinctus* (continued).

234. Fragment of a section taken just below roof of mesothorax, from a very young nymph, showing three of the six nymphal fibres at beginning of cleavage phase. A.A.T., I.H.

235. Longitudinal section of same; note distinction between myoblasts (with investing cytoplasm), and muscle nuclei. A.A.T., I.H.

236. Fragment from same nymph as figure 232, showing end-to-end fusion of three myoblasts at surface of daughter fibre. A.A.T., I.H.

237. Section along part of a developing wing-muscle fibre, from a preparation in which faint cross-striation and *Z*-membranes are perceptible. A.A.T., I.H.

238. Section along developing oblique tergal wing muscle; from half-grown nymph. A.A.T., I.H.

239. Fragment of cross-section of wing muscle, after completion of myoblast incorporation; the two rather blurred nuclei are actually muscle-fibre nuclei in mitosis. A.A.T., I.H.

Figures 240 to 250. *Drosophila melanogaster.*

240. Section along roof of mesothorax of a larva about to pupate; below epidermis is a degenerating muscle fibre, over which myoblasts are spreading. Carnoy, I.H.

241. Cross-section, rather later; shows the three transected degenerating larval muscle fibres, with investing and invading myoblasts. Carnoy, I.H.

242. Complete replacement of larval muscles by myoblasts (young pupa). Carnoy, I.H.

243. Rather later phase; the photograph represents a fragment of a cross-section, showing the crowded myoblast rudiment of the longitudinal (*d.l.m*), and oblique tergal (*o.t*) muscles. Carnoy, I.H.

244. Similar section, in which the future fibres have become defined. Carnoy, I.H.

245. Cross-section of dorsal longitudinal muscle, showing the rudiments of the future six fibres, still enclosed by unincorporated myoblasts. Carnoy, I.H.

246. Two fibres of same, late pupa. Carnoy, I.H.

247. Cross-section of a muscle fibre from a late pupa. Carnoy, I.H.

248. Section along rudiment of tergo-sternal wing muscle; young pupa. Carnoy, I.H.

249, 250. Fragments from a rather later pupa, in which fibrils are appearing within the myoblast column. Carnoy, I.H.

Plate 31

Figures 251 to 259. Cross-sections of mesothorax of various Diptera to show fibre-pattern of wing-muscle.

251. *Neoaratus hercules* (Asilidae).
252. *Neoitamus rudis* (Asilidae).
253. *Tabanus imperfectus* (Tabanidae).
254. *Trichopththalma bancrofti* (Nemestrinidae).
255. *Psychoda spatulata* (Psychodidae).
256. *Macromastix clarkiana* (Tipulidae).
257. *Oestrus ovis* (Oestridae).
258. *Syrphus viridiceps*; small (Syrphidae).
259. *Sisyromyia aurata* (Bombyliidae).

Figure 260. Enlarged fibre of *Sisyromyia aurata.*

Figures 261 to 263. *Rutilia potina* (Tachinidae).

261. Cross-section of mesothorax to show giant fibres.
262. Fragment (about fifth) of one of the fibres of the longitudinal muscle. Carnoy, I.H.
263. Fragment of same, at higher magnification, showing sarcolemma (asterisk).

Figures 264 to 268. Cross-sections of mesothorax of various Diptera, in which the wing-muscles have a constant fibre number (all to scale).

264. *Drosophila melanogaster* (Drosophilidae).
265. *Fannia canicularis* (Anthomyiidae).
266. *Stomoxys calcitrans* (Muscidae).
267. *Thelaira* sp. (Tachinidae).
268. *Calliphora stygia* (Tachinidae).

Figures 269, 270. *Calliphora stygia.*

269. Fifth and sixth fibres of dorsal longitudinal muscle. Da Fano preparation.
270. Fragment of same, higher magnification.

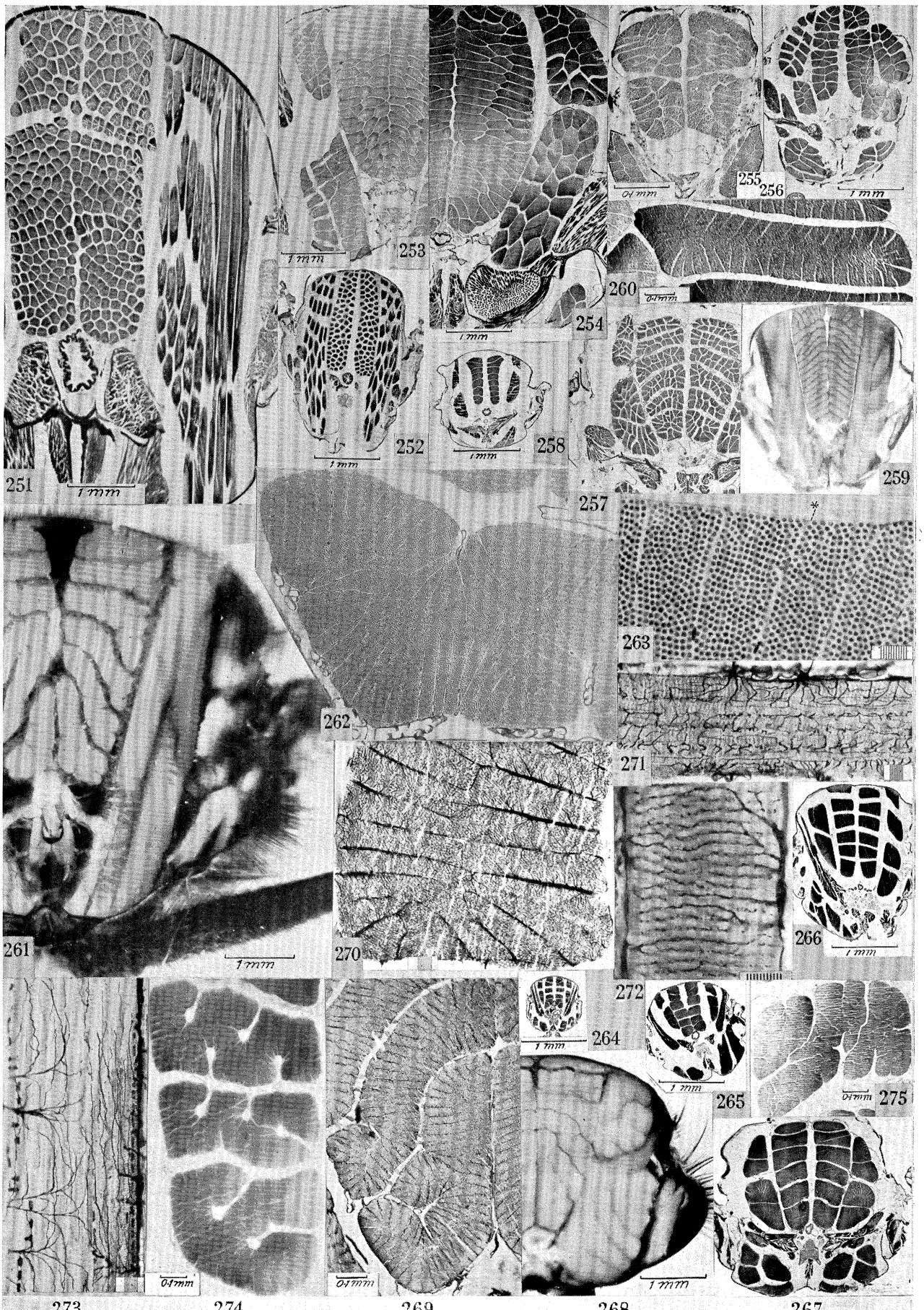
Figure 271. *Macromastix costalis* (Tipulidae), showing tracheal pattern. Da Fano preparation.

Figure 272. *Gastrophilus intestinalis* (Oestridae); tracheae. Da Fano preparation.

Figure 273. *Neoitamus rudis* (Asilidae), tracheae. Da Fano preparation.

Figure 274. Three fibres of longitudinal wing-muscle of *Eristalis tenax* (Syrphidae).

Figure 275. Fibre from *Lamprogaster laeta* (Ortalidae).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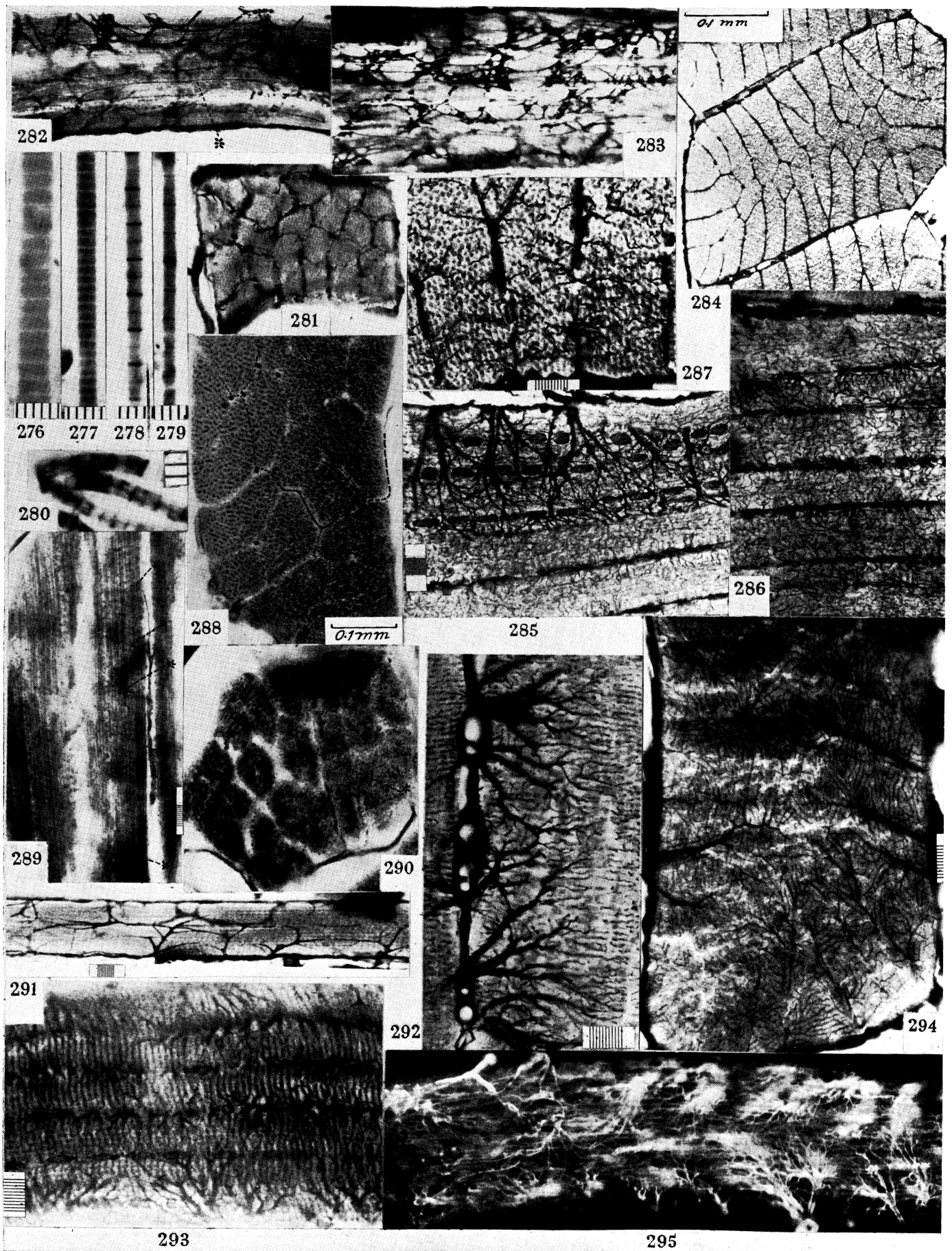
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Plate 32

Figures 276 to 279. Sarcostyles, fresh; phase contrast.

276. *Neoitamus rudis.*
277. *Chironomus duplex.*
278. *Eristalis punctulatus.*
279. *Drosophila melanogaster.*

Figure 280. Fragments of sarcostyles of *Neoitamus rudis*, showing myofibrils. Alc.B., I.H.

Figures 281 to 283. *Poecilohetaerus schineri* (Sapromyzidae). Da Fano preparation.

281. Cross-section.
282, 283. Longitudinal sections. 283 shows tracheal net within intercolumnar spaces; 282 shows (asterisk) a trachea entering sarcostyle column.

Figures 284 to 287. House-fly, showing tracheal pattern. Da Fano preparations.

284. Cross-section.
285. Longitudinal section, showing, above, large tracheae entering fibre along a cleft, within which lie also the muscle nuclei.
286. Similar section, transecting the clefts.
287. Fragment of cross-section.

Figure 288. House-fly. Cross-section of fibre prepared by Willis's silver method, showing motor-nerve ending; dotted part added from immediately adjacent sections.

Figures 289 to 290. *Drosophila*; innervation. Willis's silver method.

289. Shows anterior end of one of the muscle fibres of longitudinal muscle (partly broken), with underlying nerve, from which fine filaments enter the fibre (indicated by asterisk).
290. Cross-section, showing two nerve filaments (asterisk) entering fibre.

Figure 291. *Limnophila morula* (Tipulidae). Tracheal pattern. Da Fano preparation.

Figures 292 to 294. *Trichophthalma punctata* (Nemestrinidae). Da Fano preparations.

292. Longitudinal section along cleft, showing large entering tracheae.
293. Similar section, but transecting clefts, showing transverse path of tracheae.
294. Cross-section.

Figure 295. Wing-muscle fibre of *Culex pipiens*, showing intracellular tracheae; fresh, glycerine, dark background.

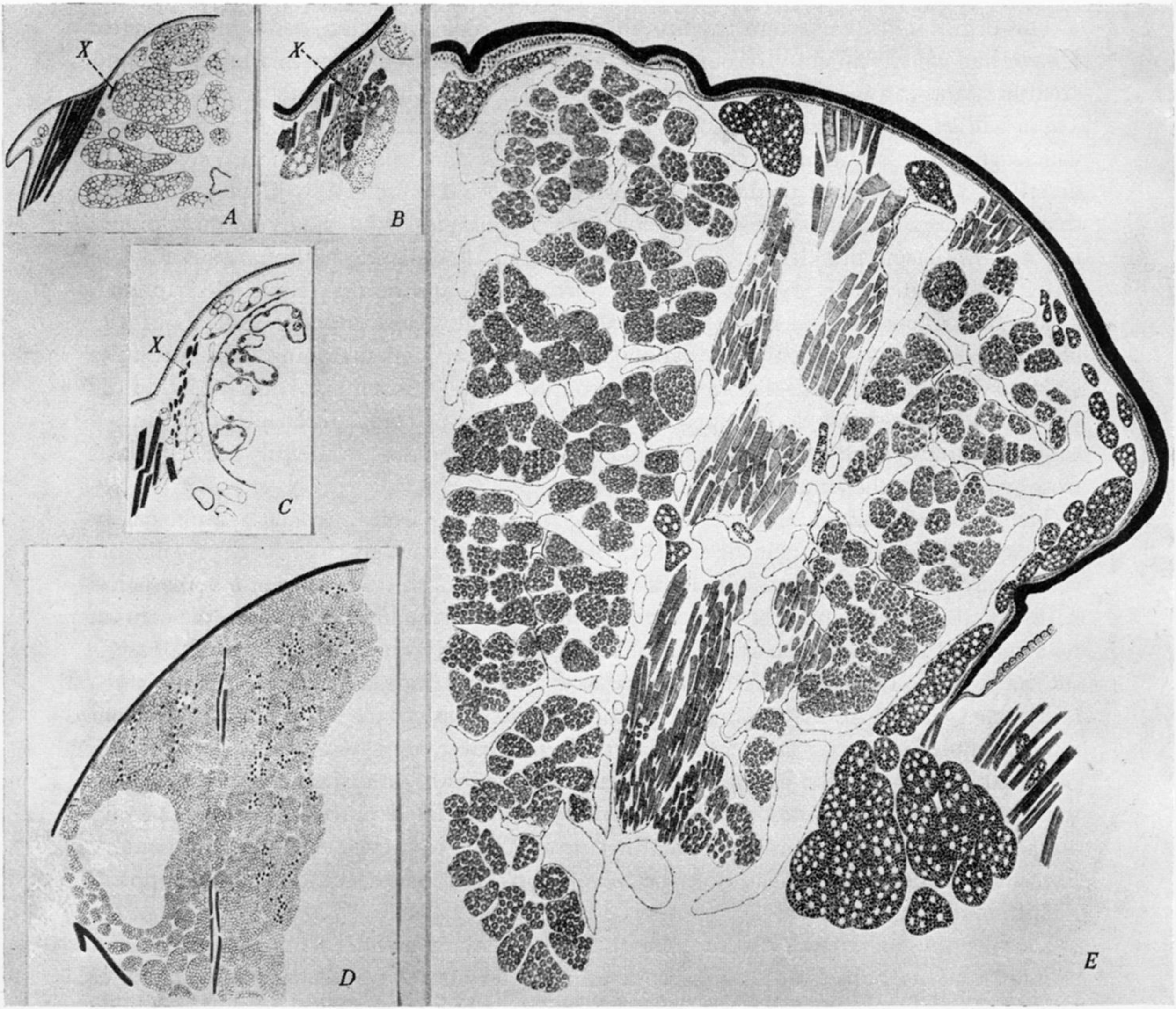
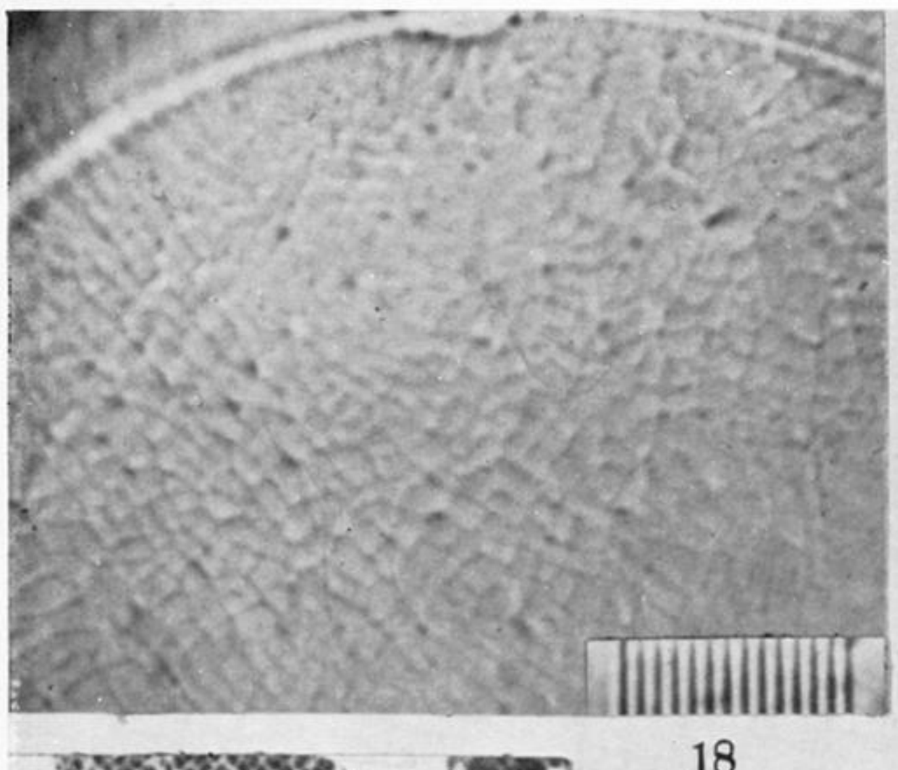


FIGURE 9. Cross-sections of mesothorax of five nymphs at successive stages of development, and drawn to scale, showing growth of dorsal longitudinal wing muscle. *A*, 10 mm nymph; *B*, 12 mm nymph; *C*, 15 mm nymph; *D*, 20 mm nymph; *E*, advanced nymph. In *A*, *B* and *C* the muscle rudiment is indicated by *X*, the labelling line in *C* pointing to the particular fragment that is shown at higher magnification in figure 157, plate 25. In *D* the rudiment has spread down into the fat-body; in *E* it is the massive wedge-shaped structure to the left. Fragments of the oblique wing muscle appear, unlabelled, in *B*, *D* and *E*.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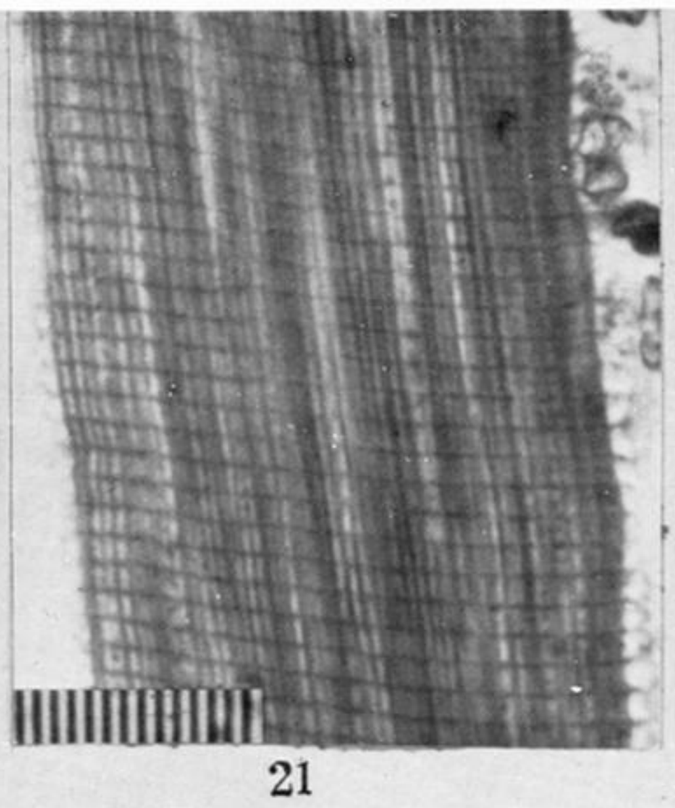
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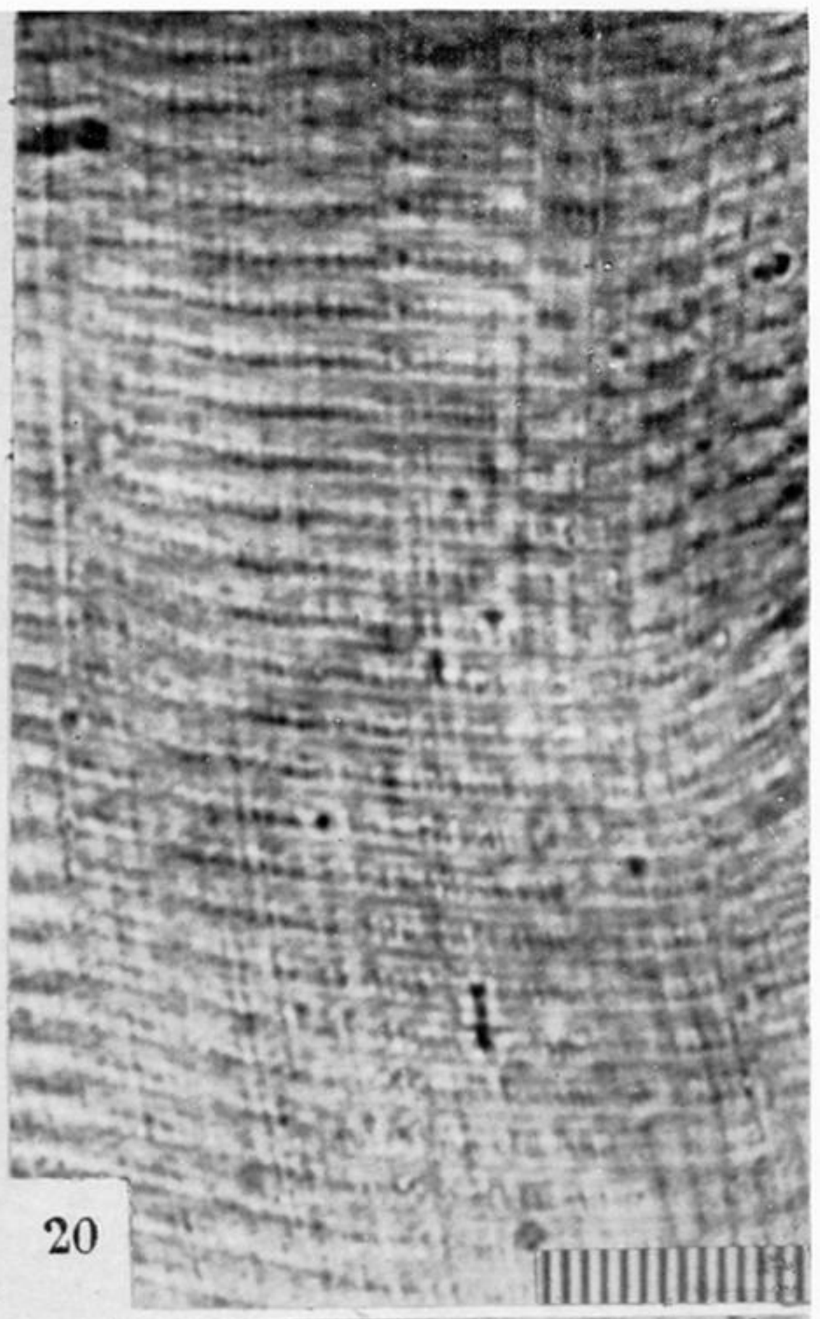
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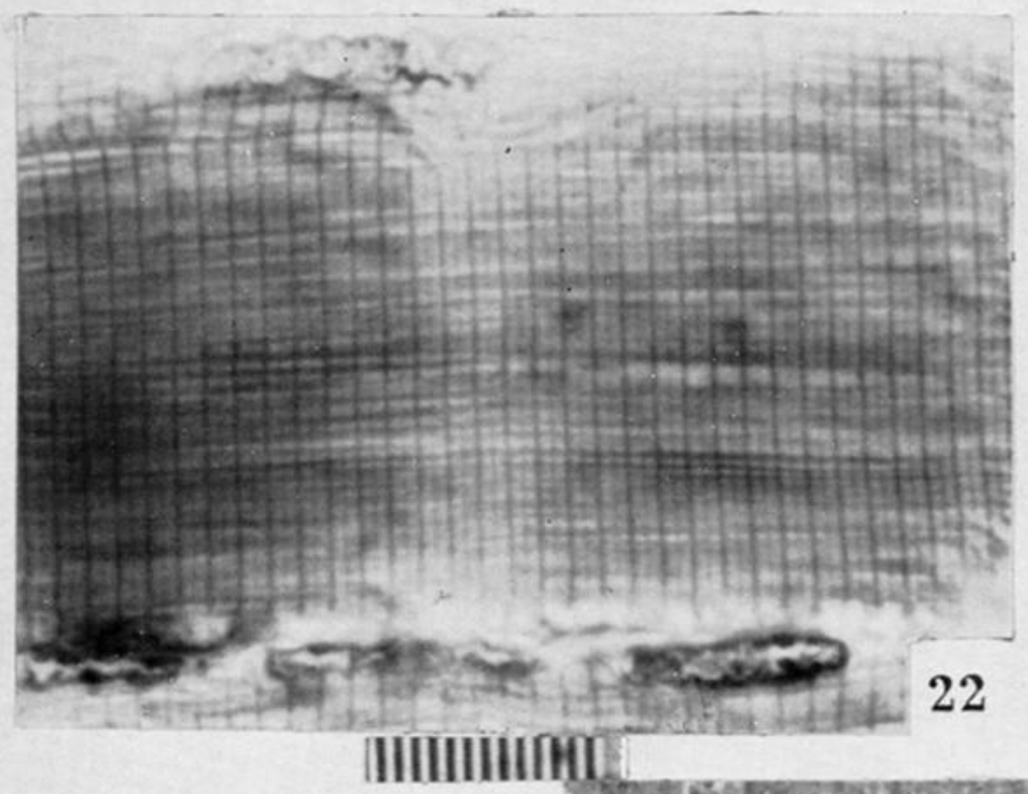
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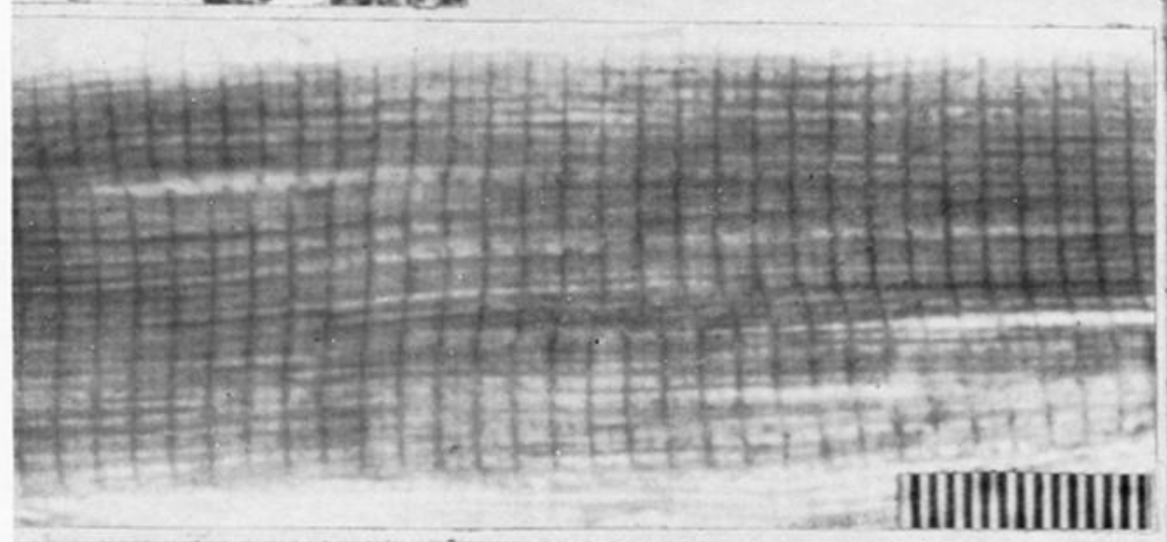
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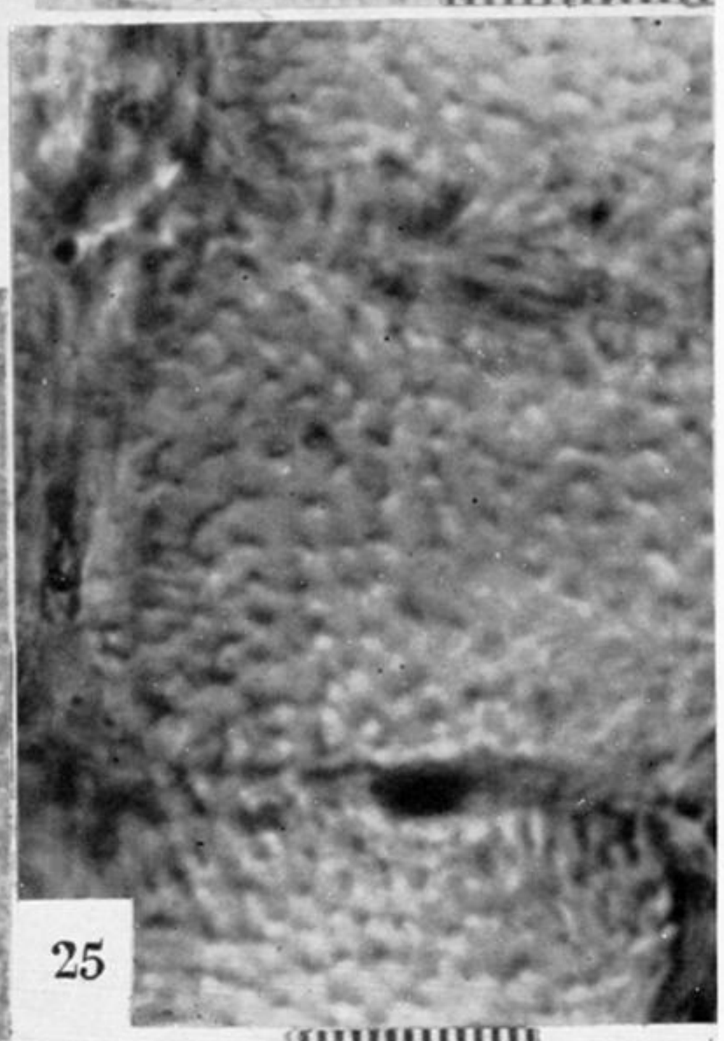
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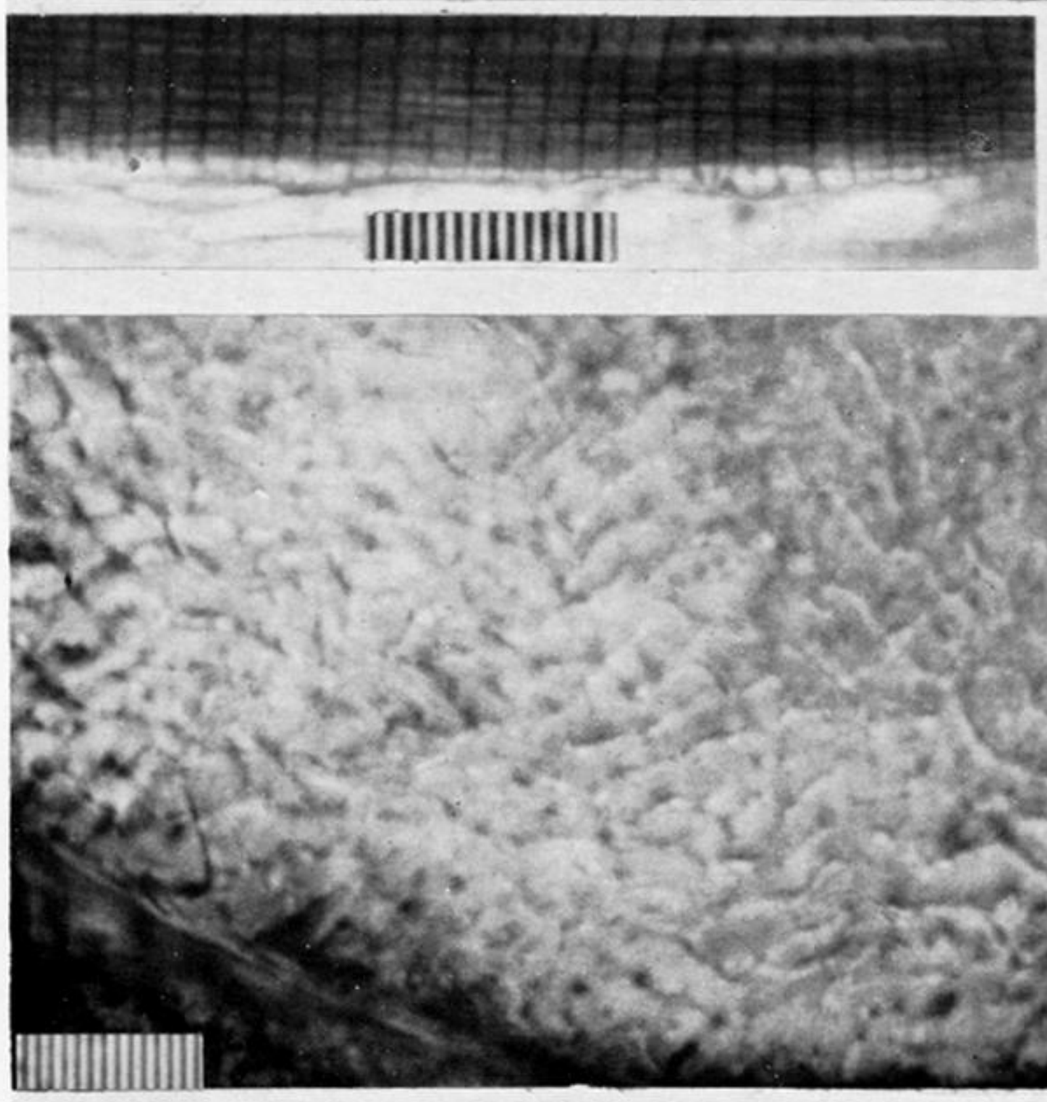
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PLATE 17

FIGURES 18 to 24. Leg muscle; rat.

18. Cross-section, showing Cohnheim's areas; fresh frozen section.
19. Cross-section, showing fibrils. Alcohol trichloracetic, I.H.
20. Longitudinal section, showing Kölliker's columns; fresh frozen section.
21. Longitudinal section, showing fibrils and *Z*-membranes. Alcohol trichloracetic, I.H.
22. Longitudinal section, showing *Z*- and *M*-membranes. Apathy's alcohol-corrosive fixative, Heidenhain's thiazine red, methylene blue stain.
23. Similar preparation, showing two 'vernier effects' of cross-membranes.
24. Fragment of similar preparation, showing cross-membrane attachment to sarcolemma.

FIGURES 25 to 27. Pectoralis major (flying) muscle of finch (*Zonaeginthus oculatus*).

25. Cross-section, showing Cohnheim areas; fresh frozen section.
26. Cross-section, showing fibril pattern. Alcohol trichloracetic, I.H.
27. Longitudinal section, showing Kölliker's columns, with sarcosomes in interstitial substance; fresh frozen section.

FIGURES 28, 29. Femoral muscle, plague locust (*Chortoicetes terminifera*).

28. Cross-section, showing Cohnheim pattern; fresh frozen section.
29. Longitudinal section, showing Kölliker's columns and sarcosomes; fresh frozen section.

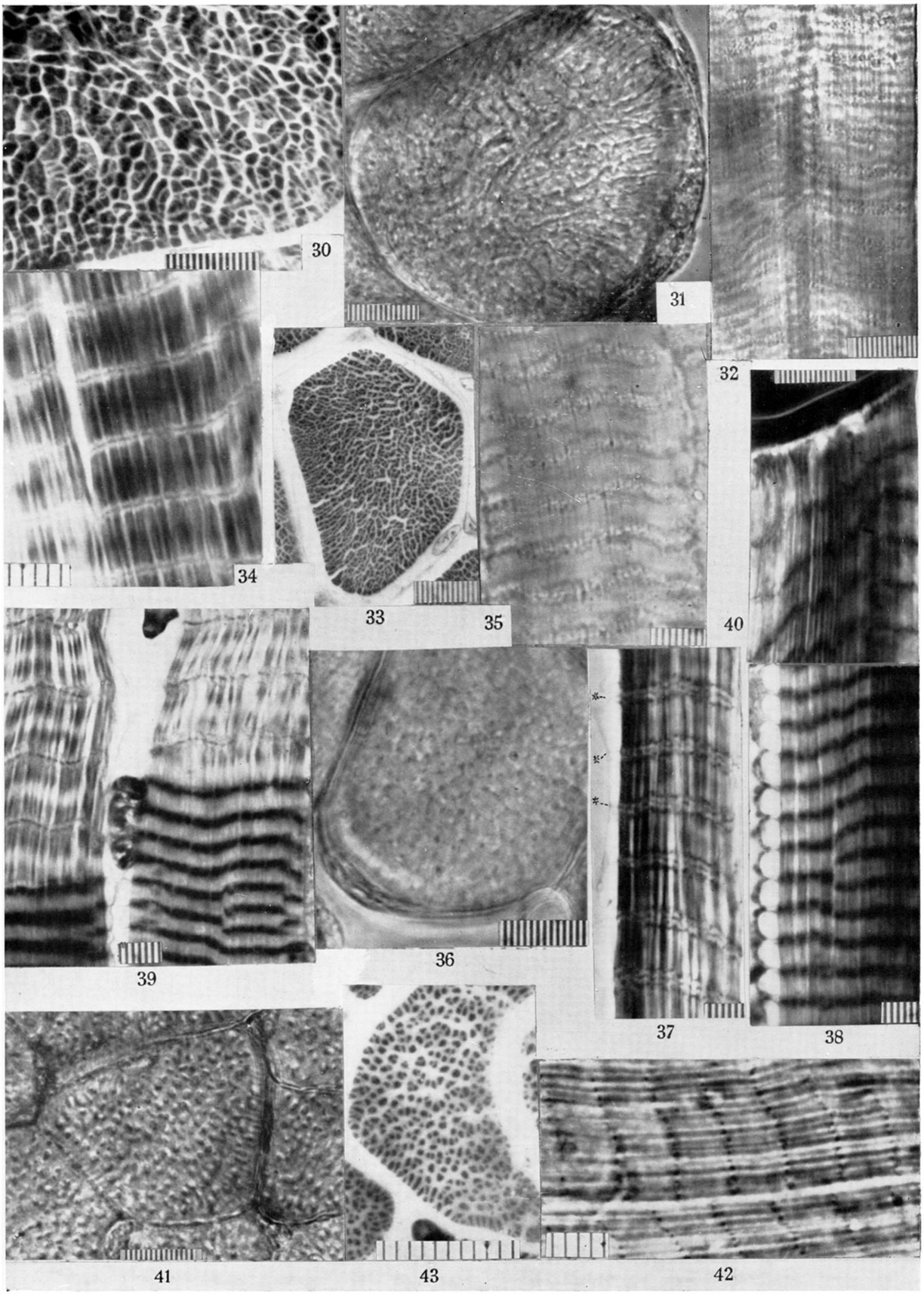

PLATE 18

FIGURE 30. Femoral muscle, *Chortoicetes terminifera*; cross-section showing sarcostyles. A.A.T., I.H.

FIGURES 31 to 34. Leg muscle, scarab larva (*Aphodius howitti*).

31. Cross-section; fresh frozen section.
32. Living muscle fibre, seen in optical section through transparent leg.
33. Cross-section showing sarcostyle pattern. Alc.B., I.H.
34. Longitudinal section, showing sarcostyles. Alc.B., I.H.

FIGURES 35 to 39. Leg muscle, cicada nymph (*Cyclochila australasiae*).

35. Longitudinal section; fresh frozen section.
36. Cross-section; fresh frozen section.
37. Longitudinal section, relaxed fibre; note 'sarcoplasmic reticulum', and, on left, attachment of *Z*-membranes to sarcolemma (marked by asterisk). Alc.B., I.H.
38. Similar preparation; contracted fibre, showing 'striation reversal'; on left, attachment of *Z*-membrane to sarcolemma.
39. Similar preparation, showing 'fixed contractile waves'.

FIGURE 40. Leg-muscle fibre—*Neoaratus hercules* (Asilidae). Focus adjusted (on right) to pass along plane of lamella; elsewhere it intersects lamellae; note resolution of lamella into subfibrils at insertion end. Alcohol formol, I.H.

FIGURES 41 to 43. Flexor mandibulae muscle (*Periplaneta americana*).

41. Cross-section; fresh frozen section.
42. Longitudinal section, showing sarcostyles; fresh frozen section, sarcoplasm removed by refrigeration.
43. Cross-section, showing compound sarcostyles. Alc.B., I.H.

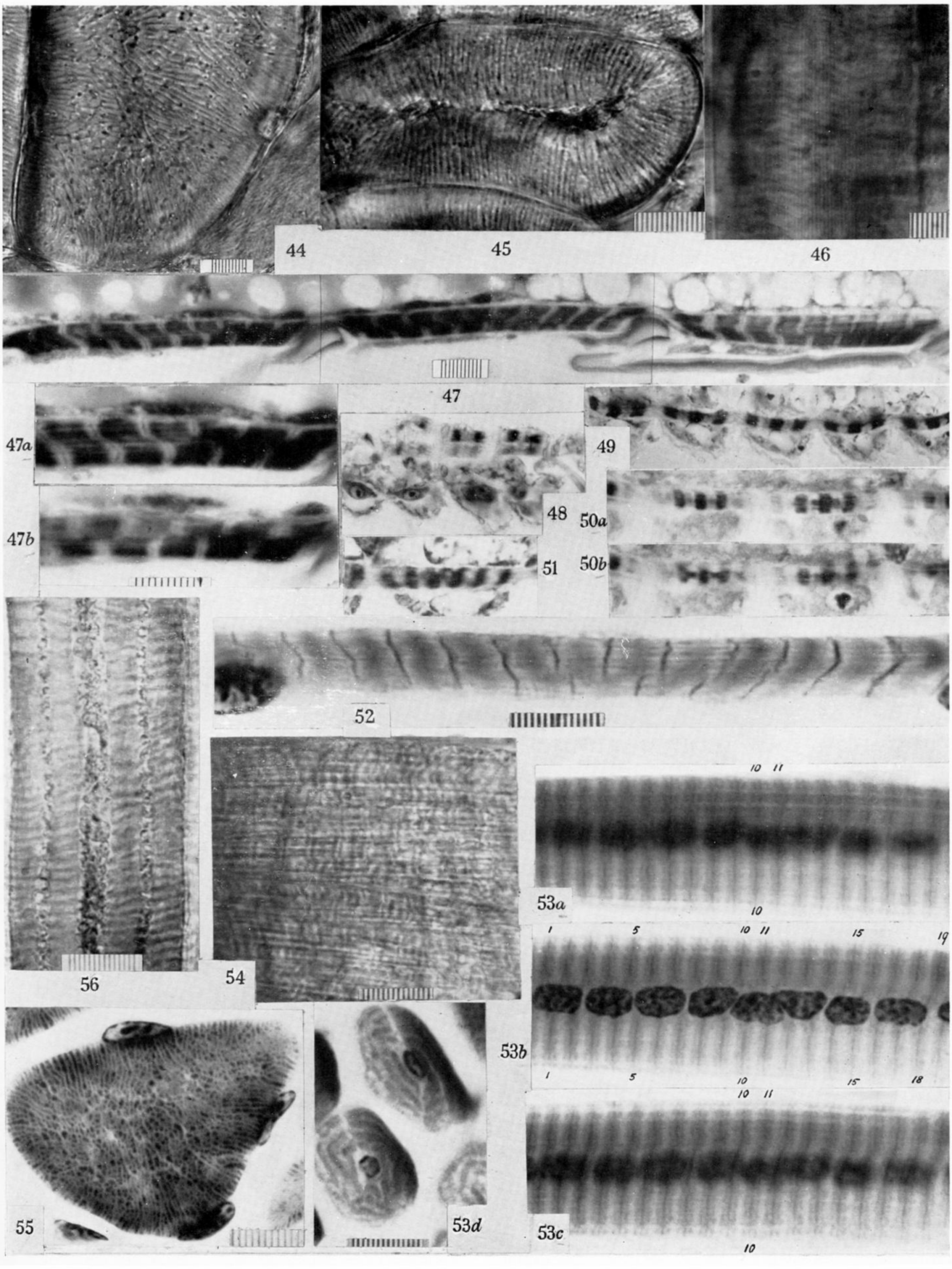

PLATE 19

FIGURE 44. Prothoracic leg-muscle fibre of grasshopper (*Caedicia olivacea*); fresh frozen section.

FIGURES 45, 46. Leg muscle of spider, *Pholcus littoralis*.

45. Cross-section; fresh frozen section.

46. Optical section along living fibre, seen through transparent leg.

FIGURES 47 to 51. Fibres of intersegmental abdominal muscle of *Thrips imaginis*, showing helicoidal striation and its development.

47. Three successive fibres, with intersegmental attachments; late nymph.

47*a*, *b*. Fragment of left-hand fibre, focused at two levels to show 'turn' of helicoid.

48. Two-striation stage, from minute nymph.

49. Three-striation stage.

50*a*, *b*. Two photographs, at very slightly different focal levels, showing transition between three- and five-striation stage, and with incipient helicoid.

51. Six-striation stage; helicoid developed.

FIGURE 52. Abdominal muscle fibre, cicada nymph, showing 'vernier'.

FIGURE 53*a–d*. Leg-muscle fibre of *Tabanus imperfectus*; *a–c*, the same fibre focused at three levels (*b*, intermediate), to show helicoidal pattern of striation; *d*, cross-section.

FIGURE 54. Living fibre of *Daphnia carinata*, seen through transparent cuticle.

FIGURE 55. Leg-muscle fibre of grasshopper, *Caedicia olivacea*; cross-section showing composite character of lamellar sarcostyles around fibre margin. Alc.B., I.H.

FIGURE 56. Living muscle fibre, house-fly; optical section. Focus passes along axial column of sarcoplasm, and intersects twice the outer concentric ring of sarcoplasm. Sarcosomes visible.

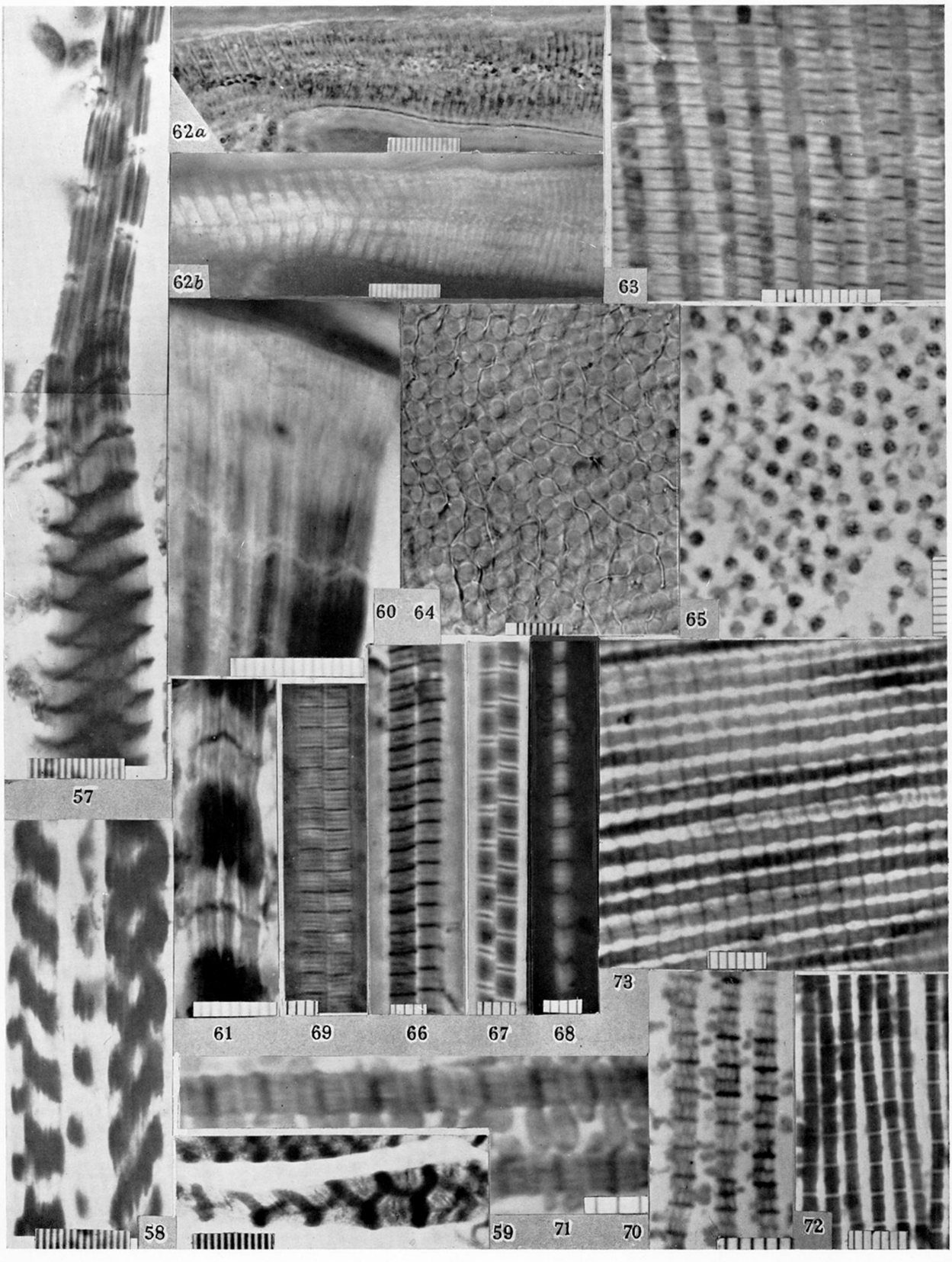

PLATE 20

FIGURE 57. Fibre of springing muscle of *Scolypopa australis* (Homoptera), showing 'fixed contractile wave' and helicoidal striation. A.A.T., I.H.

FIGURE 58. Two fibres of the same muscle; right fibre shows double helicoid; 'vernier' effect in left fibre.

FIGURE 59. Fragment of fibre of *Chrysopa* nymph, showing helicoidal striation.

FIGURE 60. Insertion end of a fibre of springing muscle of *Scolypopa australis*, photographed in ultra-violet light, with N.A. 1·95, to show composite structure of sarcostyles, and continuity of their fibrils with the tonofibrillae. A.A.T., I.H.

FIGURE 61. From the same muscle, to show character of *Z*-membrane; technique as in figure 60.

FIGURE 62*a*, *b*. Muscle fibre of house-fly, fresh, and with stationary contractile wave; *a*, with phase contrast; *b*, with polarized light and filter.

FIGURES 63 to 73. Wing muscle of bee (*Apis mellifica*).

63. Fragment of fresh fibre, phase contrast.
64. Cross-section of fresh frozen tissue; note air-filled tracheae.
65. Cross-section, showing subfibrillation of sarcostyles. Alc.B., I.H.
66. Fresh isolated sarcostyles; phase contrast.
67. The same, stretched; phase contrast.
68. Stretched fresh sarcostyle; polarized light and filter.
69. Two fresh isolated sarcostyles; phase contrast, polarized light and filter.
70. Three sarcostyles, prepared to show component myofibrils. Alc.B., I.H.
71. Sarcostyles, showing myofibrils, but with unstained *M*-membrane. A.A.T., I.H., ultra-violet light.
72. Group of sarcostyles, prepared by routine Bouin fixation, shrunken and without resolution of component fibrils.
73. Fragment of longitudinal section, showing *Z*-membrane. Carnoy, I.H.

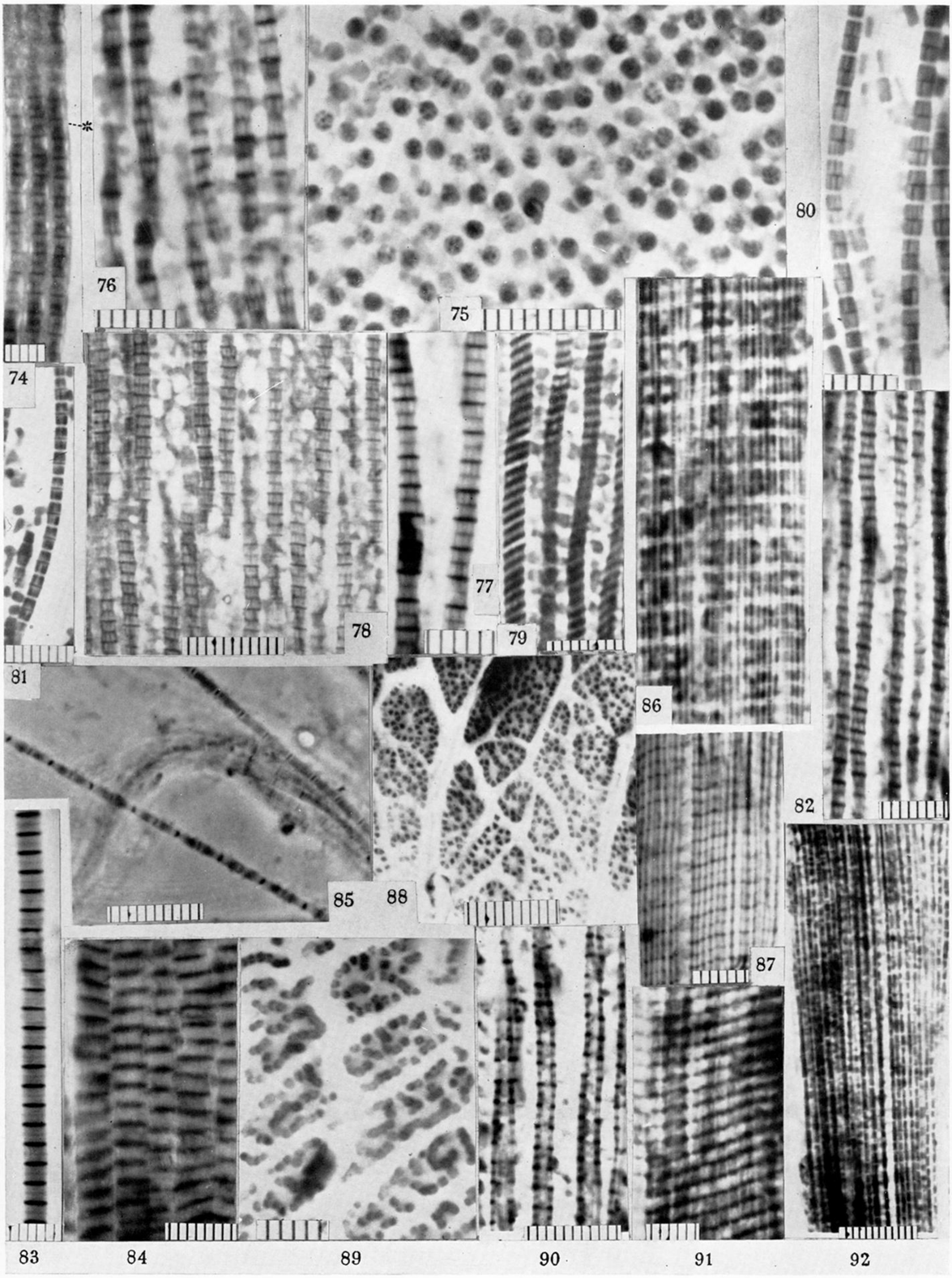

PLATE 21

FIGURE 74. *Apis mellifica*; wing muscle. Note sarcolemma bordering right margin of fibre (asterisk), with attachments at *Z*-membrane.

FIGURES 75 to 81. Wing muscle of wild bee, *Halictus speculiferus*.

75. Cross-section, showing subfibrillation of sarcostyles. Alc.B., I.H.
76. Sarcostyles, slightly stretched, showing component myofibrils. Alc.B., I.H.
77. The same, unstretched. Alc.B., I.H.
78. Fragment of a section along a fibre that has been placed for 1 h in egg-white before fixation; note *N*-granules. Alc.B., I.H.
79. Similar section, 2 h immersion before fixation; shortened sarcostyles, with 'striation reversal'. Alc.B., I.H.
80. Sarcostyles with unstained *M*-membrane. AlcB., I.H.
81. Sarcostyle, with pale band adjacent to *M*-membrane. Alc.B., I.H.

FIGURE 82. Sarcostyles from wing-muscle of *Sericesthis pruinosa* (Coleoptera). A.A.T., I.H.

FIGURES 83, 84. Sarcostyles from wing muscle of *Eurymela distincta* (Homoptera, Jassidae).

83. Fresh sarcostyle, relaxed. Phase contrast.
84. The same, shortened. Phase contrast, polarized light and filter.

FIGURES 85 to 88. Wing muscle of *Siphanta acuta* (Homoptera, Flatidae).

85. Fresh isolated sarcostyles, stretched and unstretched; phase contrast.
86. Relaxed fibre, longitudinal section; *Zf* visible, but not *Mf*; sarcosomes present. A.A.T., I.H.
87. Similar section; *Q*-band unstained, *Z*- and *M*-membranes visible. Alcohol formol trichloracetic acid, I.H.
88. Cross-section; in places there is a just perceptible subfibrillation. Alc.B., I.H.

FIGURES 89 to 91. Wing-muscle of *Cyclochila australasiae* (Homoptera, Cicadidae).

89. Fragment of cross-section, showing composite structure of sarcostyles. Alcohol formol trichloracetic, I.H.
90. Group of sarcostyles, showing component myofibrils. A.A.T., I.H.
91. Contracted sarcostyles. A.A.T., I.H.

FIGURE 92. Wing-muscle fibre of *Erythroneura ix* (Jassidae); the section is from the metathoracic tergo-sternal muscle, which has unusually thin sarcostyles. Alc.B., I.H.

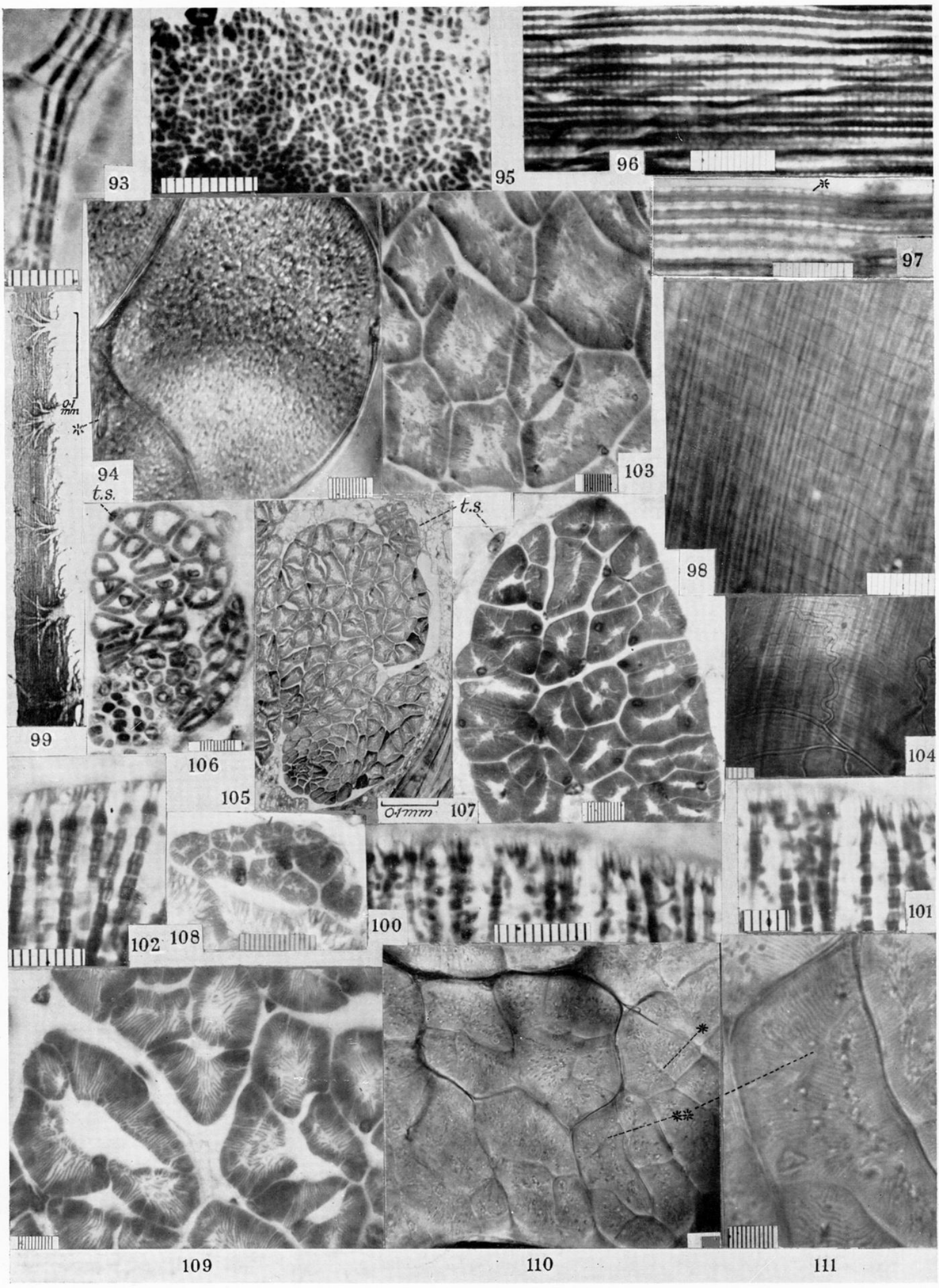

PLATE 22

FIGURES 93 to 95. Wing muscle of *Caedicia olivacea* (Orthoptera, Tettigoniidae).

93. Fresh sarcostyles; phase contrast.
94. Fresh fibre, showing Cohnheim pattern and sarcosomes; frozen section.
95. Cross-section. Alc.B., I.H.

FIGURES 96, 97. Wing muscle, *Scolia bimaculata* (Hymenoptera).

96. Showing complete *Z*- and *M*-membranes. Alc.B., I.H.
97. The same, showing their attachment to sarcolemma (asterisk).

FIGURE 98. Wing muscle of *Heteronympha merope* (Lepidoptera); fresh frozen section, showing continuous *Z*-membrane. Phase contrast, polarized light and filter.

FIGURE 99. Fragment of wing muscle of *Sisyromyia aurata* (Bombyliidae); the section grazes along the surface of the fibre, and shows tracheae within the sarcolemma. Da Fano process.

FIGURES 100, 101. Wing muscle of *Scolia bimaculata* (Hymenoptera); the section is at the muscle attachment, and shows connexion of the myofibrils of the sarcostyles with the tonofibrillae. Alc.B., I.H.

FIGURE 102. The same, from *Paracolletes* sp. Alc.B., I.H.

FIGURES 103 to 108. Wing muscle of *Blattella germanica*.

103. Cross-section of a group of typical fibres of wing musculature. Carnoy, I.H.
104. Fragment of fresh fibre, showing *Z*-membrane.
105. Cross-section of first tergo-coxal muscle of mesothorax, with attached small tergo-sternal muscle (*t.s*). Adult.
106. The same, from a 3 mm nymph.
107. Part of same, from a 6 mm nymph, showing fibre cleavage. Carnoy, I.H.
108. Developing tergo-sternal muscle from late nymph. Carnoy, I.H.

FIGURES 109 to 111. Fibre cleavage in *Periplaneta americana*.

109. Fragment of section through mesothoracic tergo-coxal muscle. Alc.B., I.H.
110. The same; frozen section of fresh tissue.
111. Fragment from figure 110, at higher magnification.

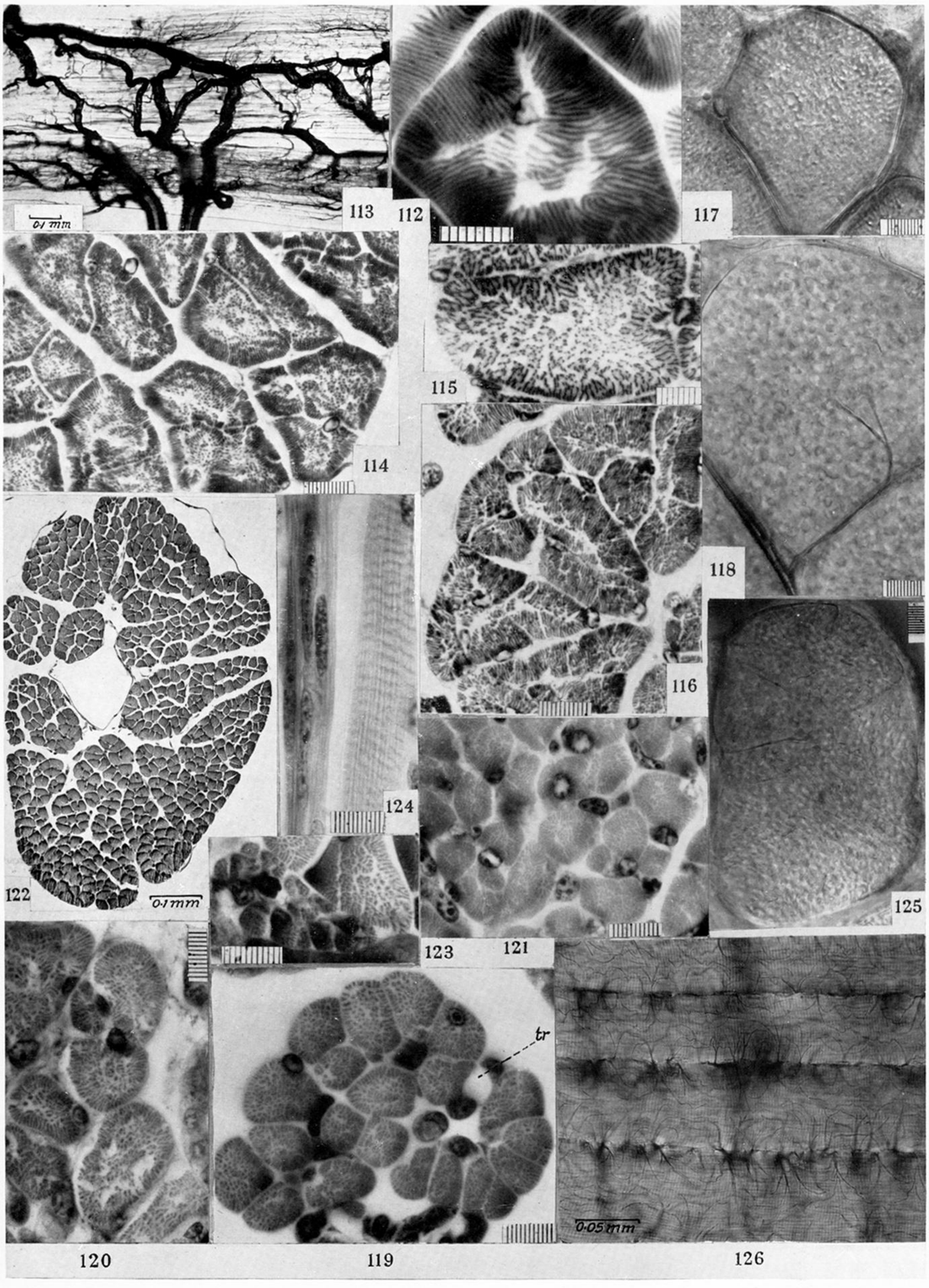

Plate 23

Figure 112. Fibre from wing musculature, *Periplaneta americana*. Alc.B., I.H.

Figures 113, 114. *Gryllotalpa australis*, wing muscle.

113. Piece of coxal remotor muscle, fresh in glycerine to show anastomosing air-filled tracheae within muscle.

114. Nymphal muscle, showing fibre cleavage. Alc.B., I.H.

Figures 115, 116. *Orthodera ministralis*, wing muscle.

115. Adult fibre. Alc.B., I.H.

116. Nymphal muscle, showing fibre cleavage. Alc.B., I.H.

Figures 117 to 124. *Acridopeza reticulata*.

117. Cross-section of mesothoracic leg muscle, female; frozen section.

118. The same, from male.

119. Cross-section of mesothoracic first tergo-coxal muscle, from 4 mm male nymph. Alc.B., I.H.; *tr*., tracheae.

120. Fragment of same, from 1 cm male nymph, showing beginning of fibre cleavage. Alc.B., I.H.

121. Fragment of same, from 15 mm nymph, cleavage advanced. Alc.B., I.H.

122. Cross-section of entire pleuro-tergal muscle of adult male.

123. The same, from a 4 mm male nymph; the large fibre alongside the group of small immature fibres is a functional leg-muscle fibre. Alc.B., I.H.

124. Longitudinal section of the same; 4 mm nymph. Alc.B., I.H.

Figures 125, 126. *Chortoicetes terminifera*.

125. Fresh fibre of wing musculature; frozen section.

126. Group of fresh fibres in glycerine, showing air-filled tracheae.

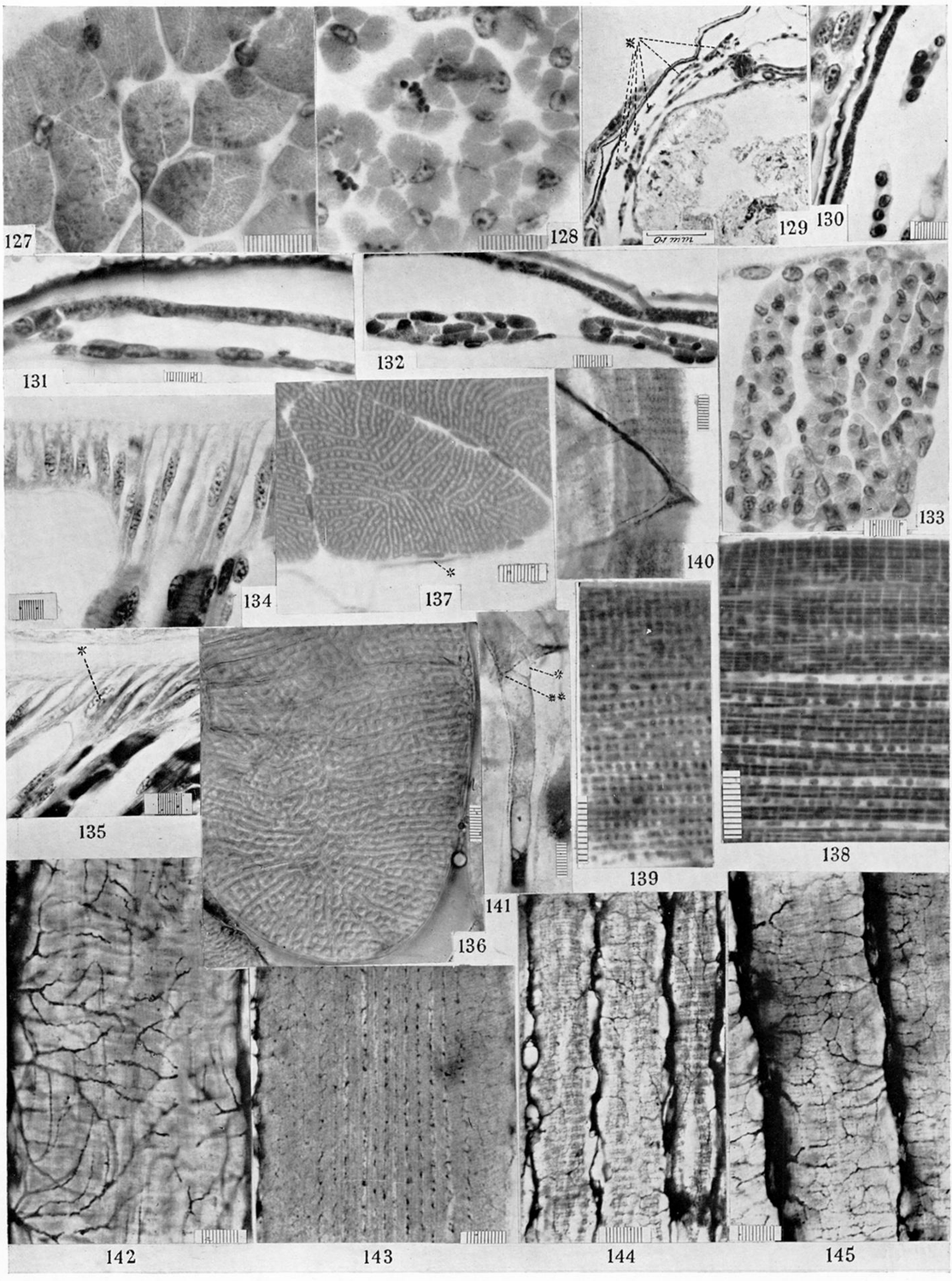

PLATE 24

FIGURES 127 to 135. *Chortoicetes terminifera.*

127. Fragment of subalar muscle, from young nymph, showing beginning of fibre cleavage. Carnoy, I.H.

128. Similar fragment, from advanced nymph, near end of cleavage phase. Carnoy, I.H.

129. Cross-section of left upper quarter of mesothorax of first instar nymph; the asterisk indicates the six rudimentary fibres from which the massive dorsal longitudinal wing muscle will develop.

130. Part of same section, at higher magnification, showing two of the rudimentary fibres. Carnoy, I.H.

131. Longitudinal section of one of the rudimentary fibres; on left is seen its attachment to overlying epidermis, above which is the chitinous covering. Carnoy, I.H.

132. Cross-section, similar to figure 130, showing beginning of fibre cleavage. Carnoy, I.H.

133. Similar cross-section from late nymph. The photograph represents one of the six fibre bundles of the dorsal longitudinal muscle, product of repeated cleavage of an original rudimentary nymphal fibre. Carnoy, I.H.

134. Section along outer margin of muscle attachment, from final instar nymph, showing recruitment of new epidermal cells into zone of attachment. Carnoy, I.H.

135. Similar section, from middle of muscle attachment, showing incipient mitosis in cell with tonofibrillae. Carnoy, I.H.

FIGURES 136 to 145. *Cyclochila australasiae*; wing muscle.

136. Adult muscle fibre, fresh frozen section; note air-filled tracheae.

137. The same, with motor-nerve ending (asterisk). Carnoy, Willis's silver process.

138. Longitudinal section; sarcosomes visible. Alc.B., I.H.

139. Similar section, showing sarcosomes. Bouin fixation, I.H.

140. Fragment of intramuscular nerve from wing musculature, showing bifurcation of the large fibre, and of accompanying delicate fibre. Carnoy, Willis's silver process.

141. Similar but thicker fragment; massive nerve fibre (one asterisk) and delicate fibre (two asterisks) branch simultaneously at point indicated by asterisks. Carnoy, Willis's silver process.

142, 143. Longitudinal sections of adult fibres, with impregnated tracheae. In figure 143 the section transects most of the lamellae, between which the tracheae lie; in figure 142 it is in the plane of the interlamellar space, and therefore shows the full expanse of the branching tracheae. Da Fano preparation.

144. Developing tracheae; the section shows three young muscle fibres, from a late nymph, into which the tracheae are growing. Da Fano preparation.

145. Later stage of same, showing development of closed tracheal net. Da Fano preparation.

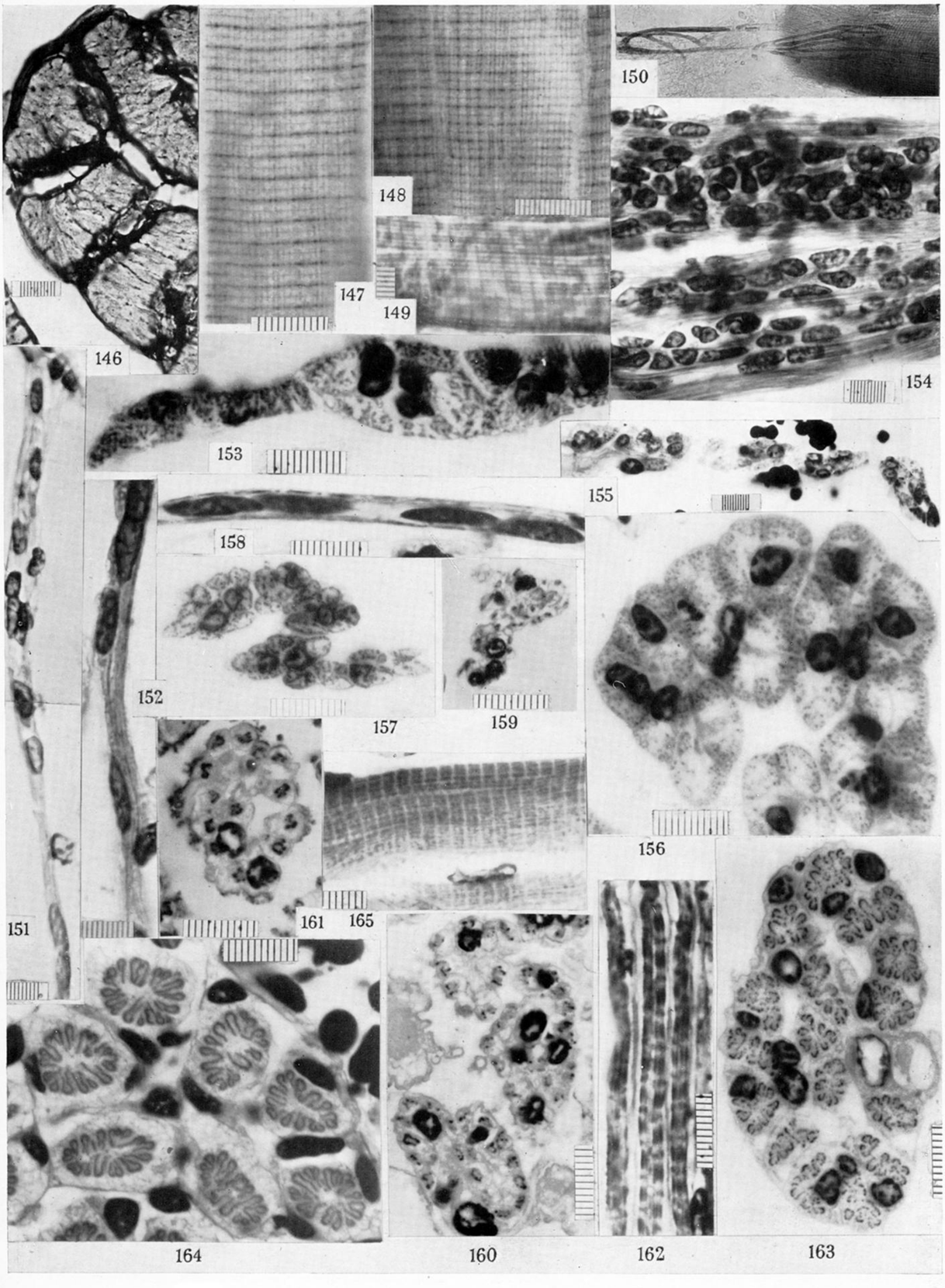

PLATE 25

FIGURES 146 to 165. *Cyclochila australasiae*, wing muscle.

146. Cross-section of a developing fibre bundle from late nymph, showing penetration of fibres by tracheae. Da Fano preparation.

147, 148. Cross-membranes in adult fibre. In figure 147 the section seems to be along a lamella; in figure 148 it plainly transects the lamellae, except in the left third of the section (for orientation see figure 138). Both figures show the *Z*-membrane, and particularly the delicate *M*-membrane, completely transecting the interlamellar spaces. Carnoy, Willis's silver process.

149. Fragment showing *Z*-membrane. Alcohol, trichloracetic acid, I.H.

150. Motor-nerve ending; gold chloride.

151. Initial stage in development of tergo-sternal muscle, showing fusion of myoblasts into a column; from a 3-day nymph. Carnoy, I.H.

152. Slightly later stage of same; fibrillation beginning, from 3-day nymph. Carnoy, I.H.

153. Cross-section of rudiment of median dorsal longitudinal muscle, from a 1 cm nymph (drawn, in position, in figure 9*a*). Carnoy, I.H.

154. Longitudinal section of same, from opposite half of same nymph.

155. Cleavage of same into five parts. Alc.B., I.H.

156. Fragment of the section shown in figure 9*b*, i.e. early in second cleavage phase. Alc.B., I.H.

157. Fragment of section shown in figure 9*c*, i.e. at end of second cleavage phase. Alc.B., I.H.

158. A single fibre from same nymph; longitudinal section.

159. Cross-section of single fibre at beginning of third cleavage phase. Alc.B., I.H.

160. Slightly later stage in development of same, showing fibril increase. Alc.B., I.H.

161. The same, showing separation of fibres in a developing fibre bundle. Alc.B., I.H.

162. Longitudinal section of same.

163. Later stage in development of fibre bundle, showing 'rosette' pattern. Alc.B., I.H.

164. Later stage of same. Alc.B., I.H.

165. Longitudinal section of fairly advanced fibre, showing 'vernier'. Alc.B., I.H.

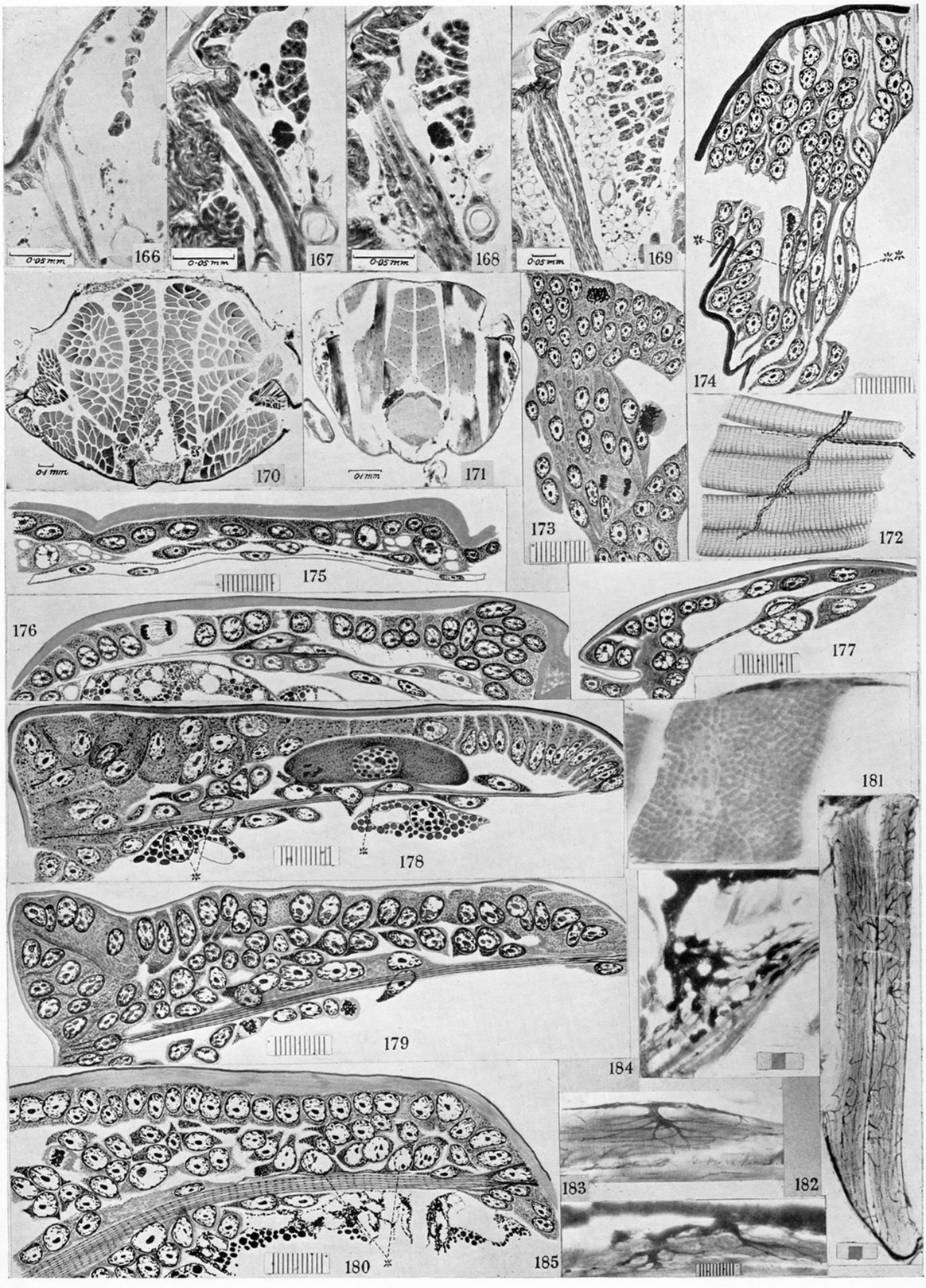

Plate 26

Figures 166 to 169. *Pauropsalta encaustica*. Cross-sections of left upper part of mesothorax of four nymphs, showing progressive development of the dorsal longitudinal muscle (transected on right) and of tergo-sternal muscle (longitudinally cut on left). Figures 166 to 168 from 6 mm nymph; figure 169 from 1 cm nymph. Alc.B., I.H.

Figure 170. *Anipo brunneus* (Jassidae); cross-section of mesothorax, adult.

Figures 171 to 185. *Erythroneura ix* (Jassidae).

171. Cross-section of mesothorax; adult.
172. Fragment of tergo-pleural muscle (wing adjustor), showing innervation. Da Fano process.
173. Early phase in development of tergo-pleural muscle. Alc.B., I.H.
174. The same, later phase.
175. Section along roof of mesothorax of a 0·7 mm nymph, showing, above the trachea, the four myoblasts from which the dorsal longitudinal muscle will develop. Alc.B., I.H.
176. Similar section, from a 0·9 mm nymph, showing pioneer myoblast. Alc.B., I.H.
177. Two-fibril stage (from oblique tergal muscle).
178. Three-fibril stage (dorsal longitudinal muscle), with three muscle nuclei (indicated by asterisk). Alc.B., I.H.
179. Seven-fibril stage. Alc.B., I.H.
180. About forty-fibril stage. Two myoblasts (marked by asterisk) are in process of incorporation into growing fibre. Alc.B., I.H.
181. Cross-section of a fibre of the dorsal longitudinal muscle, showing motor-nerve ending. Da Fano preparation.
182. Section along the two fibres that comprise the tergo-sternal (left) and tergo-coxal (right) wing muscles, showing tracheae. Da Fano preparation.
183. Tracheal end-cell, in wing-muscle fibre. Da Fano preparation.
184, 185. Two stages in development of tracheae. Figure 183 represents a section along the oblique tergal muscle, and shows invasion by tracheal cells; figure 184 shows penetration of a muscle fibre by two tracheal cells. Da Fano preparation.

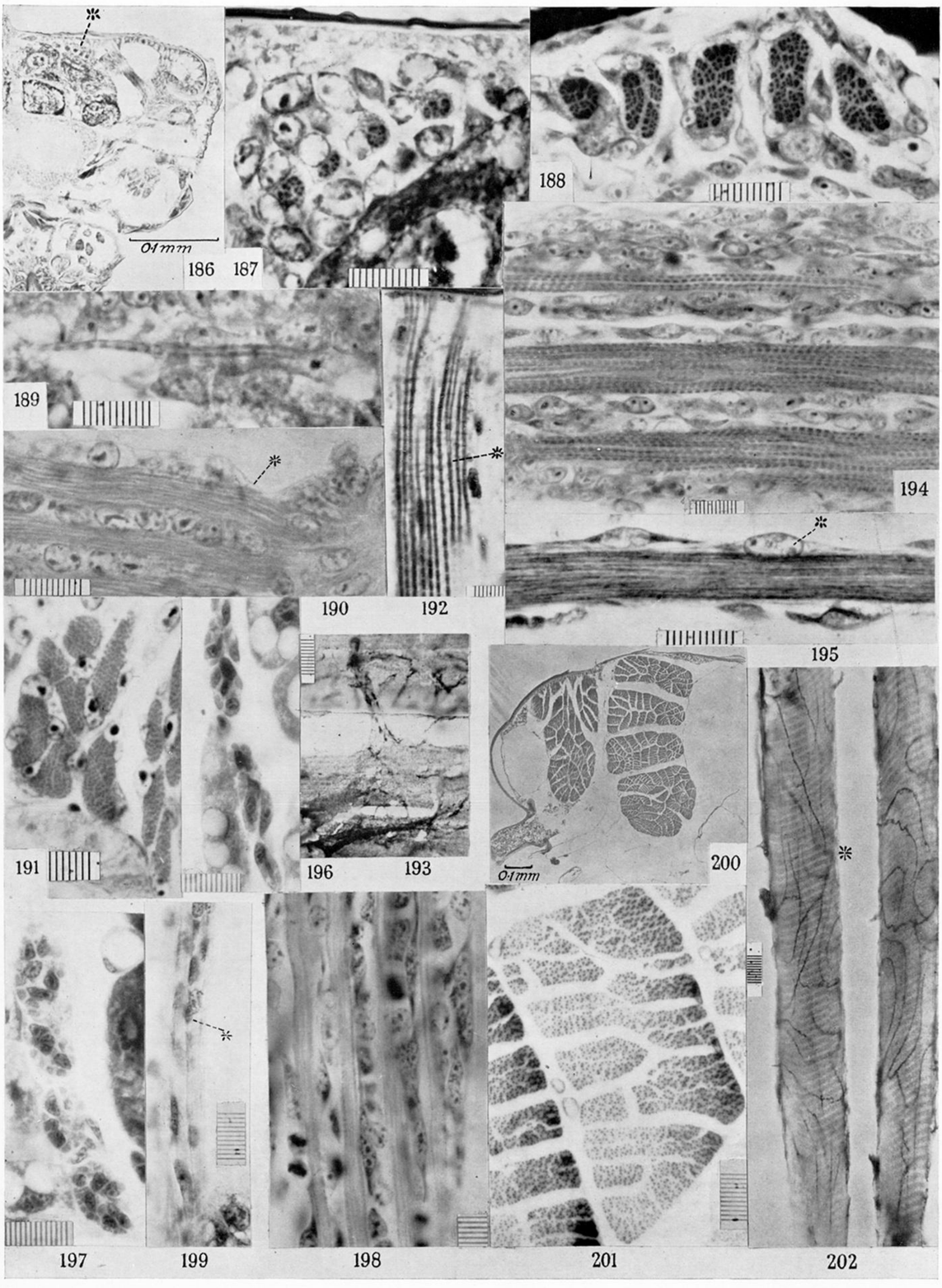

PLATE 27

FIGURES 186 to 193. *Erythroneura ix* (continued).

186. Cross-section of mesothorax of young nymph, showing rudiment of dorsal longitudinal muscle (asterisk).

187. Cross-section of muscle rudiment of same nymph (opposite side). A.A.T., I.H.

188. The same, from a more advanced nymph. Alc.B., I.H.

189. Fragment of fibre of dorsal longitudinal muscle at three-fibril stage. Carnoy, I.H.

190. Section along fibre of dorsal longitudinal muscle, from half-grown nymph, showing sarcolemma (asterisk), in continuity with basement membrane of epidermis. Alc.B., I.H.

191. Cross-section of wing-muscle fibre from late nymph, showing cleavage into fibril columns, and nuclear invasion. Alc.B., I.H.

192. Fragment of wing-muscle fibre, showing split sarcostyle. Alc.B., I.H.

193. Motor-nerve ending on wing-muscle fibre; note entrance of two separate nerve-fibres into end-organ. Da Fano preparation.

FIGURES 194, 195. *Eurinoscopus viridis* (Jassidae).

194. Section along developing wing muscle, showing free myoblasts. Alc.B., I.H.

195. Fragment from a rather earlier stage, showing, above the fibre, two myoblasts, of which one (asterisk) has elongated the full length of the photograph, and is in process of incorporation into fibre. A.A.T., I.H.; phase contrast.

FIGURES 196 to 199. *Anipo brunneus* (Jassidae).

196. Cross-section of developing dorsal longitudinal muscle, showing the five rudimentary fibres in process of cleavage. Alc.B., I.H.

197. Similar section, rather later stage. Alc.B., I.H.

198. Longitudinal section from nymph after completion of fibre cleavage, showing free myoblasts between growing fibres. Alc.B., I.H.

199. Section along fragment of same, showing myoblast filament extending along a young fibre; cell of origin indicated by asterisk.

FIGURES 200 to 202. *Scolypopa australis* (Ricaniidae).

200. Left half of adult mesothorax in cross-section, showing transected dorsal longitudinal muscle, and to left of this, part of tergo-sternal muscle. Alc.B., I.H.

201. Cross-section of single fibre of longitudinal muscle. Alc.B., I.H.

202. Section along two fibres of wing muscle, showing intracellular tracheae. Da Fano process.

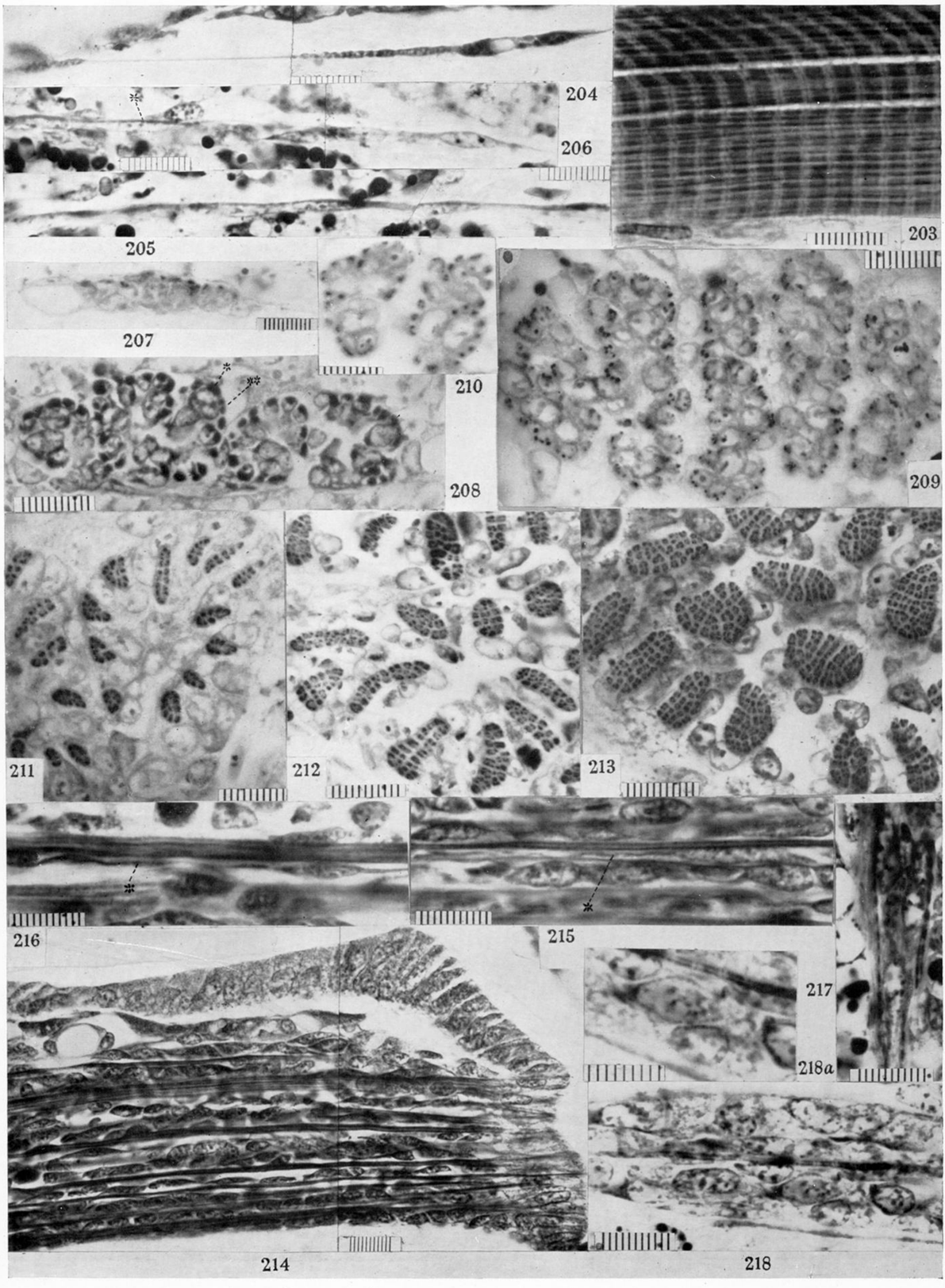

PLATE 28

FIGURES 203 to 218. *Scolypopa australis* (continued).

203. Adult wing-muscle fibre, longitudinal section. Carnoy, I.H.

204. Section along roof (hind half) of mesothorax of 1 mm nymph, showing pioneer myoblasts of dorsal longitudinal muscle. Alc.B., I.H.

205. Section along an initial muscle-fibre rudiment, from a rather larger nymph (dorsal longitudinal muscle). Alc.B., I.H.

206. Adjacent section from same, showing free myoblasts, and a fragment of fibre with cross-striation.

207. Cross-section from rather later nymph, showing incipient cleavage; the minute black dots are transected fibrils. Trachea to left. Alc.B., I.H.

208. Similar section, but from an older nymph, showing cleavage of muscle rudiment into five columns (fragment of trachea to right). Alc.B., I.H.

209. Later stage of same, fibre cleavage completed. A.A.T., I.H.

210. Fragment from a slightly earlier stage, showing single fibril stage in development of future wing muscle fibre. A.A.T., I.H.

211. Cross-section of part of muscle rudiment, showing definitive fibres considerably more advanced than in figure 209.

212. Similar section, later stage. A.A.T., I.H.

213. Similar section, still later stage. A.A.T., I.H.

214. Section along anterior third of developing dorsal longitudinal wing muscle, from young nymph, showing free myoblasts among developing fibres. A.A.T., I.H.

215. Fragment of same, to show filamentous outgrowth from myoblast.

216. Another fragment, showing end-to-end fusion of myoblasts; myoblast to left not quite focused.

217. Fragment of longitudinal section of stage shown in figure 210; note one- and two-fibril stages, with cross-striation. A.A.T., I.H.

218. Similar section, showing two-fibril stage. A.A.T., I.H.

218*a*. Fragment of figure 218, enlarged to show Z-membrane.

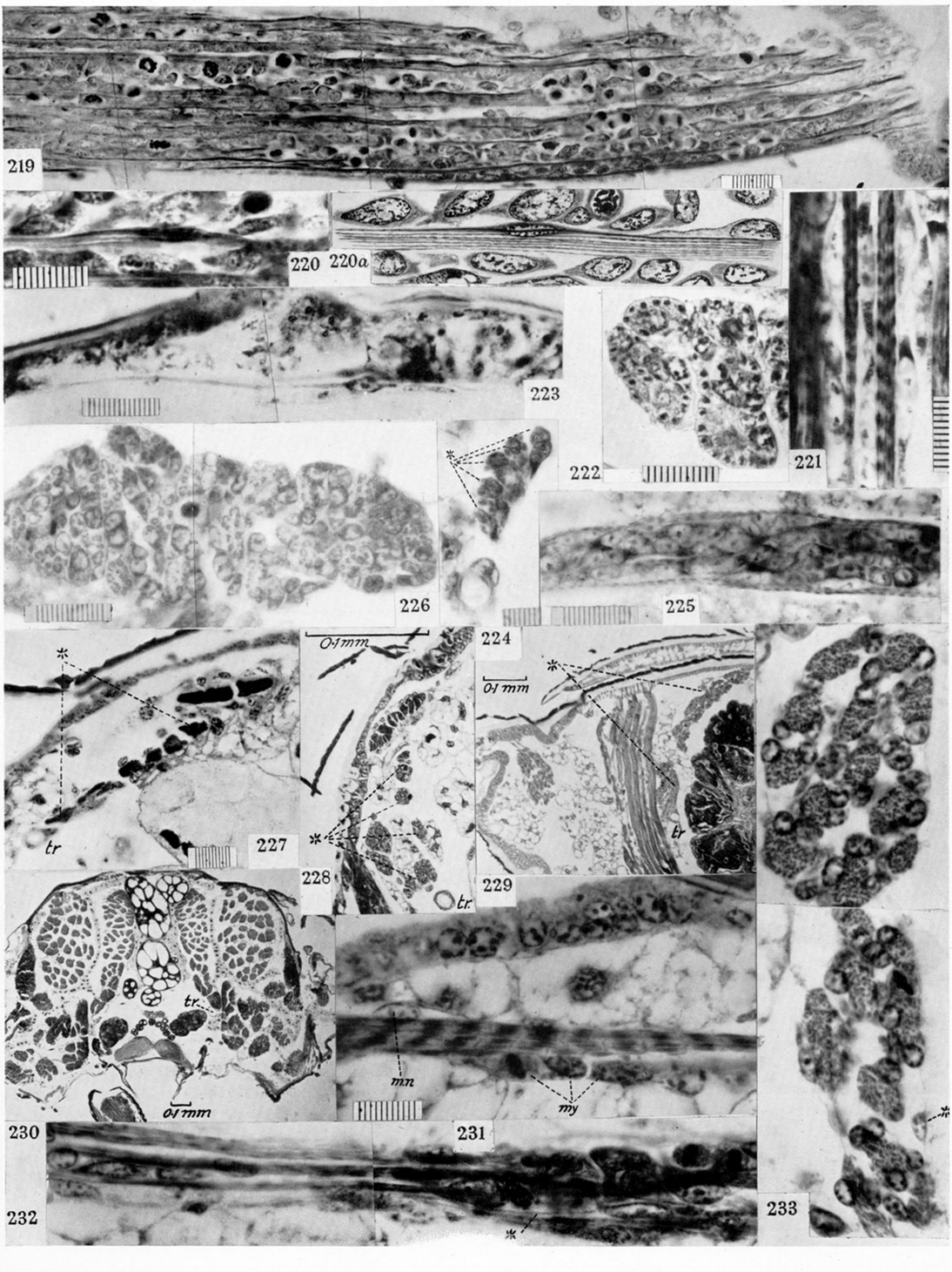

PLATE 29

FIGURES 219 to 222. *Scolypopa australis* (continued).

219. Section along developing dorsal longitudinal muscle (anterior half) at a stage rather earlier than that of figure 214, most of the fibres being at the one- to four-fibril stage (cf. figure 209); note intense mitotic activity among myoblasts. A.A.T., I.H.

220, 220*a*. Fragment showing incorporation of formerly free myoblast into a young muscle fibre; in order to bring out the full length of the myoblast the drawing (figure 220*a*) has been made, making full use of focus adjustment.

221. Fragment of a longitudinally cut developing fibre, showing helicoidal striation; note free myoblasts to side of fibre. (From same nymph as figure 212.)

222. Cross-section of muscle rudiment at about stage shown in figure 209; poor resolution of fibrils, but good definition of sarcolemma. Alc.B., I.H.

FIGURES 223 to 226. *Perkinsiella saccharicida* (Delphacidae).

223. Section along roof of thorax of minute nymph, showing a single pioneer myoblast. A.A.T., I.H.

224. Cross-section of muscle rudiment, from a minute nymph, showing initial cleavage into five parts. The five rudimentary fibres are indicated by asterisk; below are some free myoblasts. Trachea below muscle rudiment. A.A.T., I.H.

225. Section along one of the five developing fibre columns, from a rather later nymph, showing, marginally, the young fibres, enclosing a core of crowded myoblasts. A.A.T., I.H.

226. Cross-section of developing dorsal longitudinal muscle of half-grown nymph, showing early stage of fibre enlargement. A.A.T., I.H.

FIGURES 227 to 233. *Bathylus albicinctus* (Cercopidae).

227. Cross-section of mesothorax of minute nymph, taken just median to left wing base (not included). The six parent fibres of the future dorsal longitudinal wing muscle are indicated by asterisk. *tr.* trachea. A.A.T., I.H.

228, 229. Similar section from two older nymphs, showing growth of wing musculature. *tr.* trachea. A.A.T., I.H.

230. Cross-section of mesothorax, adult female. *tr.* trachea.

231. Section along roof of mesothorax of young nymph, showing one of the six nymphal fibres cut longitudinally; note distinction between muscle nuclei (*m.n*) and myoblasts (*my*).

232. Section along developing wing muscle from a young nymph. The section, which represents about half the length of the muscle, shows a bundle of four daughter fibres, progeny of a single nymphal fibre. On extreme left are three muscle nuclei and two myoblasts. In right half of photograph are nuclei and myoblasts (one in mitosis), but mostly too crowded for observation. From one of the myoblasts (asterisk) a filamentous outgrowth has formed. A.A.T., I.H.

233. Part of cross-section, shown at low magnification in figure 229; the section shows the progeny of the third and fourth nymphal fibres, cleavage about two-thirds complete.

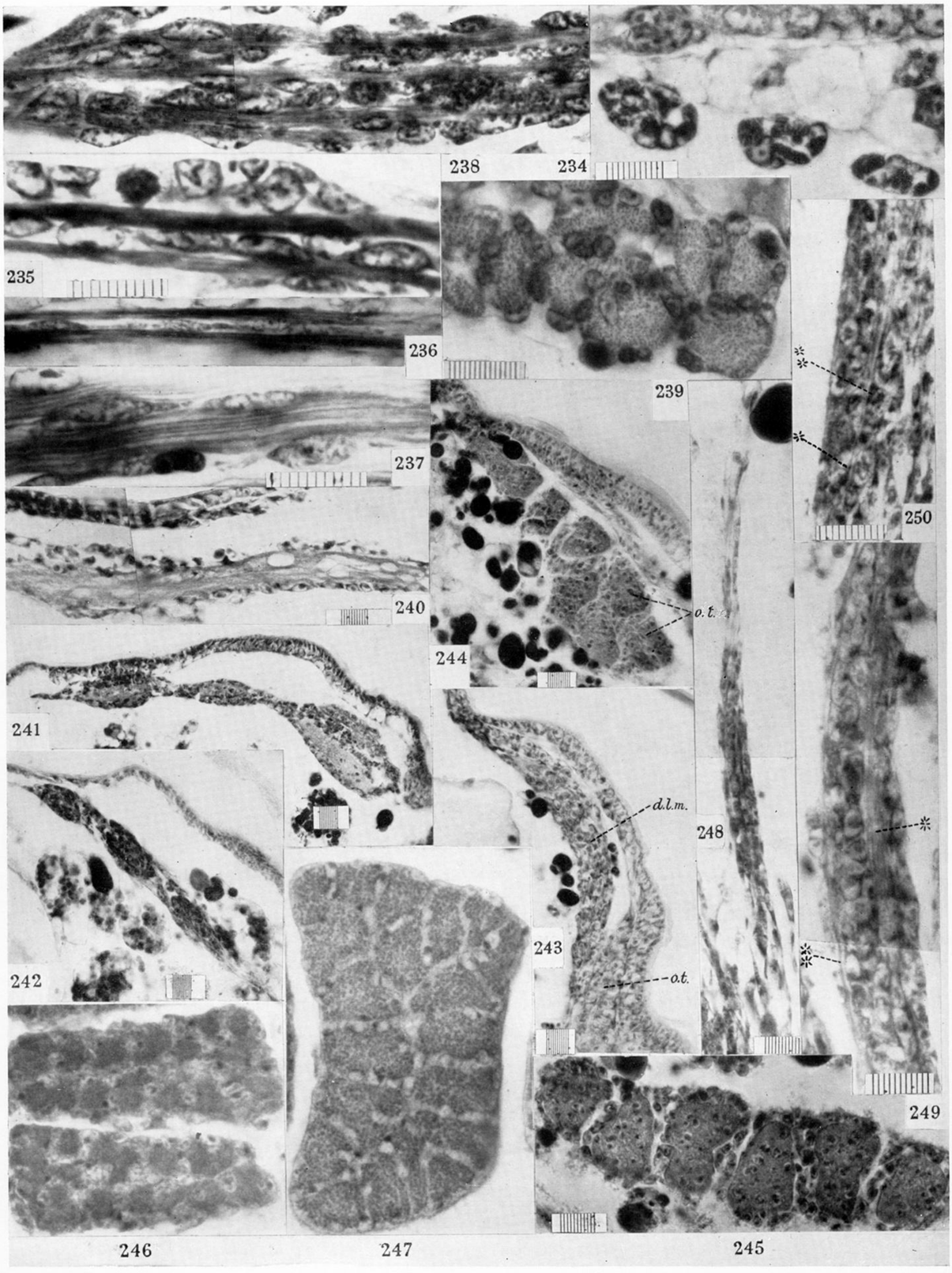

PLATE 30

FIGURES 234 to 239. *Bathylus albicinctus* (continued).

234. Fragment of a section taken just below roof of mesothorax, from a very young nymph, showing three of the six nymphal fibres at beginning of cleavage phase. A.A.T., I.H.

235. Longitudinal section of same; note distinction between myoblasts (with investing cytoplasm), and muscle nuclei. A.A.T., I.H.

236. Fragment from same nymph as figure 232, showing end-to-end fusion of three myoblasts at surface of daughter fibre. A.A.T., I.H.

237. Section along part of a developing wing-muscle fibre, from a preparation in which faint cross-striation and *Z*-membranes are perceptible. A.A.T., I.H.

238. Section along developing oblique tergal wing muscle; from half-grown nymph. A.A.T., I.H.

239. Fragment of cross-section of wing muscle, after completion of myoblast incorporation; the two rather blurred nuclei are actually muscle-fibre nuclei in mitosis. A.A.T., I.H.

FIGURES 240 to 250. *Drosophila melanogaster*.

240. Section along roof of mesothorax of a larva about to pupate; below epidermis is a degenerating muscle fibre, over which myoblasts are spreading. Carnoy, I.H.

241. Cross-section, rather later; shows the three transected degenerating larval muscle fibres, with investing and invading myoblasts. Carnoy, I.H.

242. Complete replacement of larval muscles by myoblasts (young pupa). Carnoy, I.H.

243. Rather later phase; the photograph represents a fragment of a cross-section, showing the crowded myoblast rudiment of the longitudinal (*d.l.m*), and oblique tergal (*o.t*) muscles. Carnoy, I.H.

244. Similar section, in which the future fibres have become defined. Carnoy, I.H.

245. Cross-section of dorsal longitudinal muscle, showing the rudiments of the future six fibres, still enclosed by unincorporated myoblasts. Carnoy, I.H.

246. Two fibres of same, late pupa. Carnoy, I.H.

247. Cross-section of a muscle fibre from a late pupa. Carnoy, I.H.

248. Section along rudiment of tergo-sternal wing muscle; young pupa. Carnoy, I.H.

249, 250. Fragments from a rather later pupa, in which fibrils are appearing within the myoblast column. Carnoy, I.H.

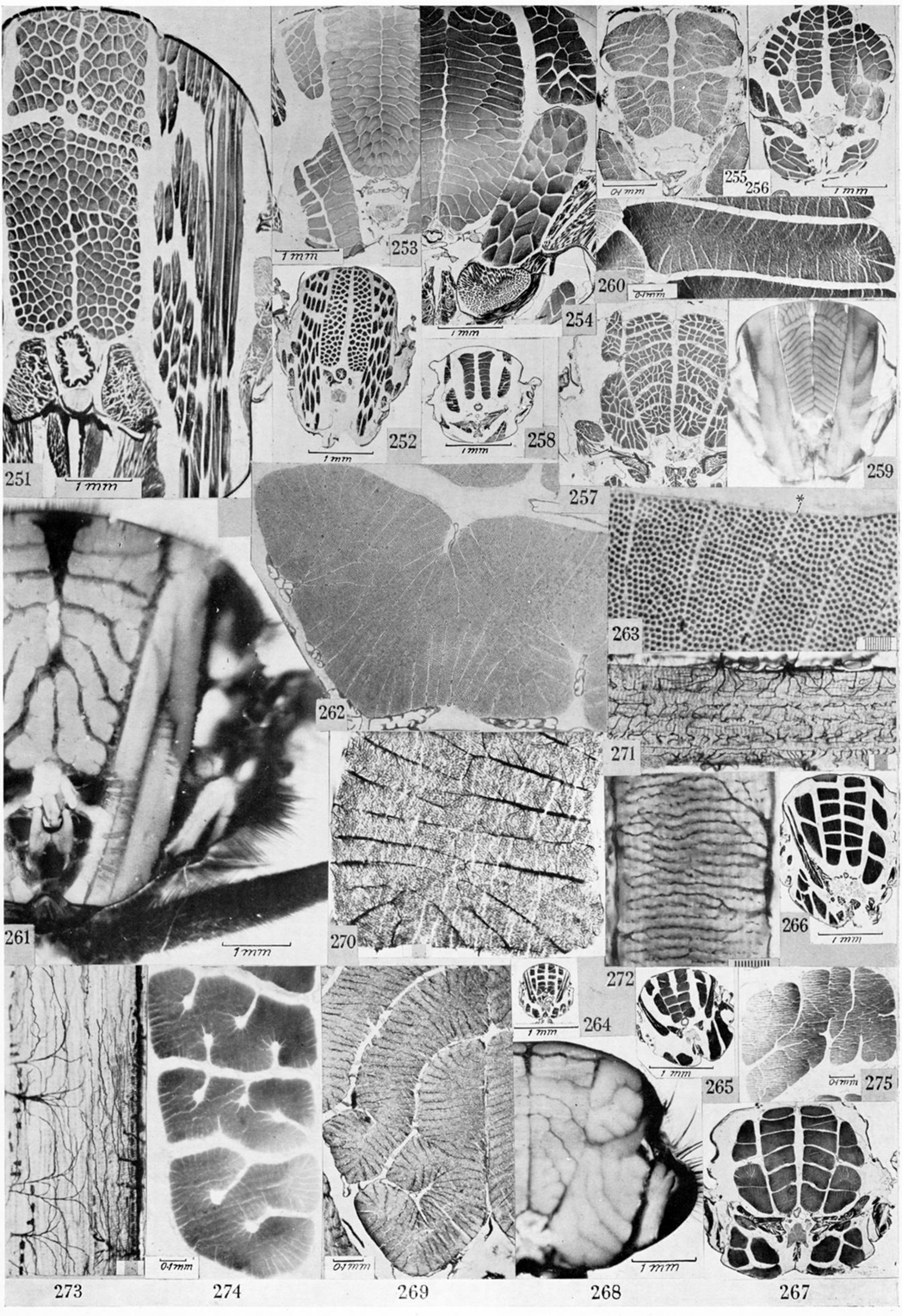

PLATE 31

FIGURES 251 to 259. Cross-sections of mesothorax of various Diptera to show fibre-pattern of wing-muscle.

251. *Neoaratus hercules* (Asilidae).
252. *Neoitamus rudis* (Asilidae).
253. *Tabanus imperfectus* (Tabanidae).
254. *Trichopthalma bancrofti* (Nemestrinidae).
255. *Psychoda spatulata* (Psychodidae).
256. *Macromastix clarkiana* (Tipulidae).
257. *Oestrus ovis* (Oestridae).
258. *Syrphus viridiceps*; small (Syrphidae).
259. *Sisyromyia aurata* (Bombyliidae).

FIGURE 260. Enlarged fibre of *Sisyromyia aurata*.

FIGURES 261 to 263. *Rutilia potina* (Tachinidae).

261. Cross-section of mesothorax to show giant fibres.
262. Fragment (about fifth) of one of the fibres of the longitudinal muscle. Carnoy, I.H.
263. Fragment of same, at higher magnification, showing sarcolemma (asterisk).

FIGURES 264 to 268. Cross-sections of mesothorax of various Diptera, in which the wing-muscles have a constant fibre number (all to scale).

264. *Drosophila melanogaster* (Drosophilidae).
265. *Fannia canicularis* (Anthomyiidae).
266. *Stomoxys calcitrans* (Muscidae).
267. *Thelaira* sp. (Tachinidae).
268. *Calliphora stygia* (Tachinidae).

FIGURES 269, 270. *Calliphora stygia*.

269. Fifth and sixth fibres of dorsal longitudinal muscle. Da Fano preparation.
270. Fragment of same, higher magnification.

FIGURE 271. *Macromastix costalis* (Tipulidae), showing tracheal pattern. Da Fano preparation.

FIGURE 272. *Gastrophilus intestinalis* (Oestridae); tracheae. Da Fano preparation.

FIGURE 273. *Neoitamus rudis* (Asilidae), tracheae. Da Fano preparation.

FIGURE 274. Three fibres of longitudinal wing-muscle of *Eristalis tenax* (Syrphidae).

FIGURE 275. Fibre from *Lamprogaster laeta* (Ortalidae).

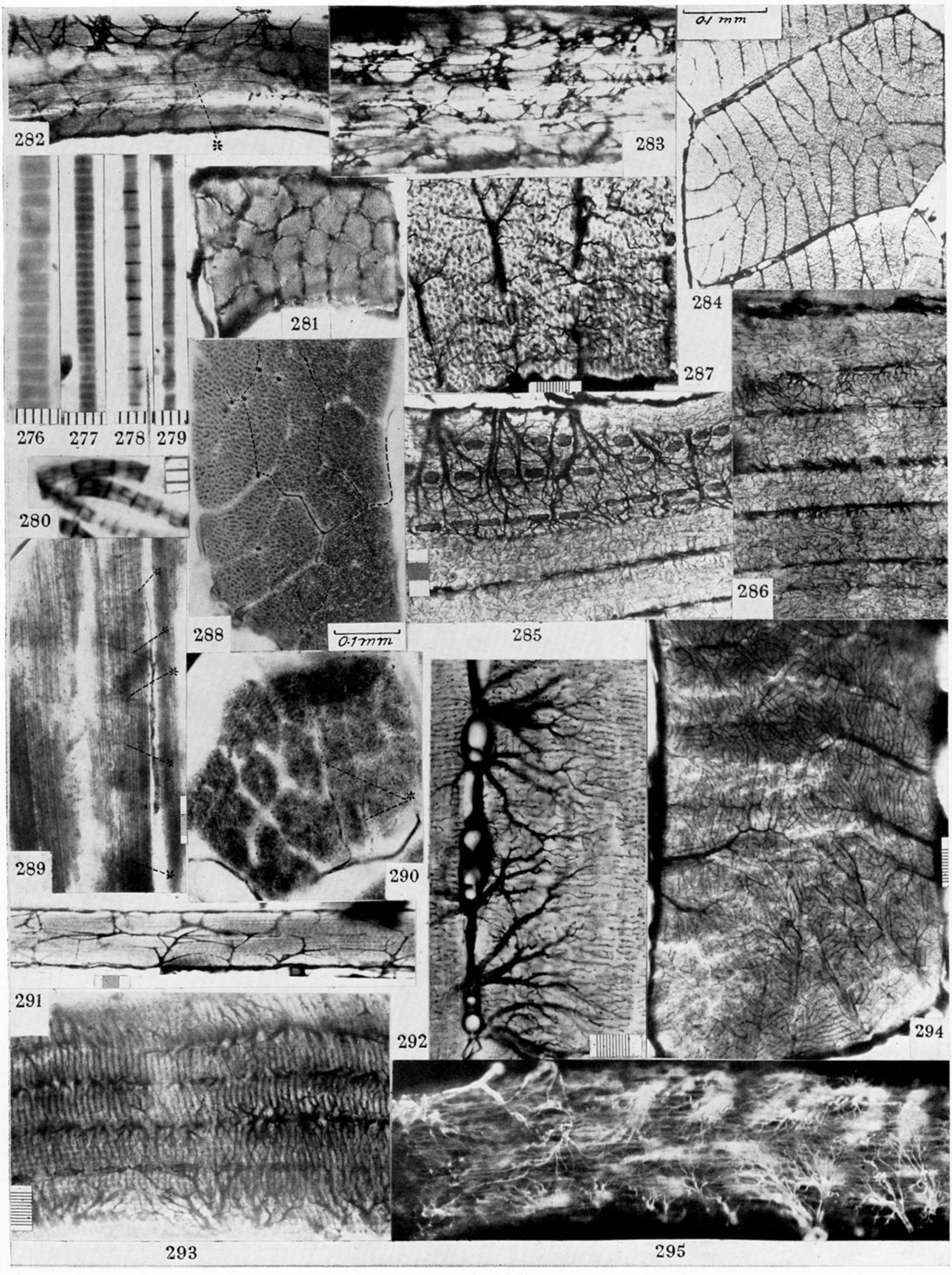

PLATE 32

FIGURES 276 to 279. Sarcostyles, fresh; phase contrast.

276. *Neoitamus rudis*.
277. *Chironomus duplex*.
278. *Eristalis punctulatus*.
279. *Drosophila melanogaster*.

FIGURE 280. Fragments of sarcostyles of *Neoitamus rudis*, showing myofibrils. Alc.B., I.H.

FIGURES 281 to 283. *Poecilohetaerus schineri* (Sapromyzidae). Da Fano preparation.

281. Cross-section.
282, 283. Longitudinal sections. 283 shows tracheal net within intercolumnar spaces; 282 shows (asterisk) a trachea entering sarcostyle column.

FIGURES 284 to 287. House-fly, showing tracheal pattern. Da Fano preparations.

284. Cross-section.
285. Longitudinal section, showing, above, large tracheae entering fibre along a cleft, within which lie also the muscle nuclei.
286. Similar section, transecting the clefts.
287. Fragment of cross-section.

FIGURE 288. House-fly. Cross-section of fibre prepared by Willis's silver method, showing motor-nerve ending; dotted part added from immediately adjacent sections.

FIGURES 289 to 290. *Drosophila*; innervation. Willis's silver method.

289. Shows anterior end of one of the muscle fibres of longitudinal muscle (partly broken), with underlying nerve, from which fine filaments enter the fibre (indicated by asterisk).
290. Cross-section, showing two nerve filaments (asterisk) entering fibre.

FIGURE 291. *Limnophila morula* (Tipulidae). Tracheal pattern. Da Fano preparation.

FIGURES 292 to 294. *Trichophthalma punctata* (Nemestrinidae). Da Fano preparations.

292. Longitudinal section along cleft, showing large entering tracheae.
293. Similar section, but transecting clefts, showing transverse path of tracheae.
294. Cross-section.

FIGURE 295. Wing-muscle fibre of *Culex pipiens*, showing intracellular tracheae; fresh, glycerine, dark background.