STUDIES ON CERTAIN ASPECTS OF THE NEUROMUSCULAR PHYSIOLOGY OF INSECTS.

PART 1.

The significance of the sheath surrounding the peripheral nerves in phytophagous and carnivorous insects.

PART 2.

Studies on the histology, and certain aspects of the neuromuscular physiology of the flight muscles in *Nudaurelia cytherea capensis* Stoll.

by

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CONTENTS.

Page No.

PART I.

THE SIGNIFICANCE OF THE SHEATH SURROUNDING THE PERIPHERAL NERVES IN PHYTOPHAGOUS AND CARNIVOROUS INSECTS.

PART II.

STUDIES ON THE HISTOLOGY, AND CERTAIN ASPECTS OF THE NEUROMUSCULAR PHYSIOLOGY OF THE FLIGHT MUSCLES IN NUDAURELIA CYTHEREA CAPENSIS STOLL.

PART 1.

THE SIGNIFICANCE OF THE SHEATH SURROUNDING THE PERIPHERAL NERVES IN PHYTOPHAGOUS AND CARNIVOROUS INSECTS.

RESUMÉ.

It is well known that high potassium ion concentrations depolarize nervous tissue and it has been suggested that the nerve sheath surrounding the peripheral nerves of insects serves as a protective barrier for the exclusion of potassium ions, in the haemolymph, from the immediate environment of axons.

Further it is known that the concentration of potassium ions in the haemolymph of phytophagous insects is far higher than that in predatory forms; this has led to the suggestion that the nerve sheath in plant feeding insects should be more highly developed than that of entomophagous insects. In this work the structure of the nerve sheath in phytophagous and predatory insects has been studied and this assumption has been shown to be groundless.

However, preliminary experiments on the effects of ions and drugs on the peripheral nerves of phytophagous and predatory insects have shown that there is a definite difference in susceptibility between the nerves of these two forms and this has led to the postulate of a diffusion barrier beneath the level of the nerve sheath, which is more highly developed in phytophagous than in predatory forms. The properties of this second barrier are discussed.

PART 1.

INTRODUCTION.

While in recent years our knowledge of the neuromuscular physiology of insects has grown rapidly (recent reviews of the subject include those of Hoyle 1957, Roeder 1953, Roeder 1958, and Vowles 1961), information upon the pharmacology of these animals is extensive but much of it is unsatisfactory (Crozier 1922, Crozier and Pilz 1924, Hamilton 1939, Roeder and Roeder 1939, Roeder 1958, Roeder and Weiant 1948, Twarog and Roeder 1957, Harlow 1958, Voskresenkaya 1945, Vereshtchagin et al. 1961, Hill and Usherwood 1961, and Narahashi 1962 a and b). One reason for this position is that in phytophagous and omnivorous insects, the normally chosen experimental material, there exists a thick sheath around the nerves. Hoyle (1953) and Twarog and Roeder (1956) working on the peripheral nerves of the locust and the central nervous system of the cockroach respectively, have shown that a high concentration of potassium depolarizes the insect nervous system. In both cases removal of the surrounding nerve sheath resulted in a great increase in the rate of depolarization of the nerves. It has been suggested (Hoyle 1953 and Yamasaki and Narahashi 1959, 1960) that this sheath serves as a protective barrier to exclude potassium ions in the haemolymph from the immediate environment of the axons.

Hoyle has further suggested that a similar but as yet unidentified barrier must protect the muscle fibres and probably also the myoneural junctions from the ions in the haemolymph. These barriers, essential for the normal function of the neuromuscular system in insects characterised by high blood potassium, probably also serve to exclude pharmacological agents from the reactive surfaces of nerves, muscles and junctions.

These considerations led to the postulate that in entomophagous insects this barrier might be far less developed since it is known that in these forms the blood potassium concentration is far lower than in phytophagous and omnivorous insects (Muttkowski 1923, Boné 1944 and 1946, Tobias 1948 a and b, Florkin 1949, and Roeder 1953. My own determinations on the potassium and sodium ion concentrations in the blood of the predatory bug Ectrichodia crux are in agreement with these observations. In this case the potassium concentration in the haemolymph is very low, the sodium to potassium ratio being about 10 : 1). If this were the case the possibility was envisaged that entomophagous insects would be particularly suitable for pharmacological studies. The first section of this thesis is dedicated to a study of this point. It will be shown that in fact the sheath is as well developed in entomophagous as in other insects, but that the barriers to protect the nerves against the effects of excess potassium ions are less well developed in entomophagous forms.

MATERIAL AND CULTURE METHODS.

In selecting material for such an investigation it is essential that the insects chosen should be both reasonably abundant and large. Phytophagous Orthoptera are readily obtained and in the present work both Locusta migratoria migratorioides R and F from the laboratory stock and Maphyteus leprosus Fab. have been used. The latter were collected as last instar nymphs and maintained for several months in laboratory cages. They were fed on fresh grass every two or three days and their condition appeared to be better than that of adults freshly collected from the field. Specimens of Zonoceros elegans (Thunb.), Dictyophorus spumans (Thunb.), Acanthacris ruficornis (Fab.) were also occasionally used. Periplaneta americana L. was taken as an example of a typically omnivorous form. This material was originally collected in an East London bakery and the laboratory stock maintained in plastic dishes. The animals were fed biscuits and occasionally minced meat. On this diet they survived well and for long periods.

The choice of entomophagous insects is more limited and only two species were found which satisfied the requirements both of size and abundance. These were the mantid, Sphodromantis gastrica (Stal.), and the reduviid Ectrichodia crux (Thunb.). The latter was fairly readily obtainable in the field in early winter. These reduviids live well in plastic dishes in the laboratory and have been kept for over six months during which time they oviposited. They survive best when they are given rubble beneath which they could crawl. Ectrichodia feeds readily on millipedes and the adults were normally fed about once a fortnight. Stocks of millipedes were maintained for this purpose and survived well with almost no attention. Sphodromantis is more difficult to maintain in the laboratory. They were kept individually in plastic dishes at 26°C. Adults were reared successfully from oothecae collected in the field. The early instars were fed on Drosophila adults. The task of collecting suitable live insects as food for the larger individuals was tedious and often difficult.

ANATOMY AND HISTOLOGY.

In a comparison of the barriers between the haemolymph and the nerves and muscles of these insects, the first question which may be asked is whether the perilemma of the two entomophagous insects chosen for study is significantly thinner than that of the phytophagous forms and omnivorous forms such as Periplaneta.

To answer this question it is clearly desirable to study the structure of homologous nerves. Since, further, it had been the intention to study the pharmacology of neuromuscular transmission of the entomophagous forms, should the barriers against the haemolymph prove to be less effective in these animals, attention was particularly directed to the leg muscles as these provide convenient and familiar material for physiological study. Hoyle (1955 a,b) has shown that in Locusta the metathoracic leg receives double innervation from nerves 3b and 5. (This follows Pringle's (1939) nomenclature in Periplaneta). A similar pattern of innervation is found in Periplaneta (Dresden and Nijenhuis 1953, 1958, Nijenhuis and Dresden 1952, 1955). I have made a series of dissections both to confirm these observations and to determine the organisation of the nerves supplying the metathoracic legs of the other insects chosen for study. Such material was fixed for about 30 minutes in Alcoholic Bouin immediately before use and dissected under 90% alcohol. Dissection of the femoral nerves and muscles was found to be facilitated by shaving the chitin from the ventral surface of the limb, fixing for at least six hours in 90% alcohol and subsequently staining the material for a few minutes in dilute methyl-blue eosin. As the dissection proceeded the new surfaces exposed were stained. My dissections confirm the findings of Hoyle and Dresden and Nijenhuis upon Locusta and Periplaneta and show that this arrangement is found also in Maphyteus, Sphodromantis and Ectrichodia Plates 1.1, 1.2 and 1.3.

The recognition of two nerves entering the coxa does not, however, tell us which corresponds to nerve 3b and which to nerve 5 of Locusta and Periplaneta. A provisional homology may be established by assuming that the nerve arising more anteriorly from the metathoracic ganglion corresponds to nerve 3. Some support is given to this assumption by the fact that the more anterior nerve is invariably finer than the more posterior one (Table 1.1).

TABLE 1.1.

Animal.	Nerve	Nerve Major Axis (μ)	Nerve Minor Axis (μ)
Maphyteus	5	180	125
Periplaneta	5	234	180
Sphodromantis	5	107	100
Ectrichodia	5	96	75
Maphyteus	3b	74	57
Periplaneta	3b	65	46
Sphodromantis	3b	54	43
Ectrichodia	3b	52	48

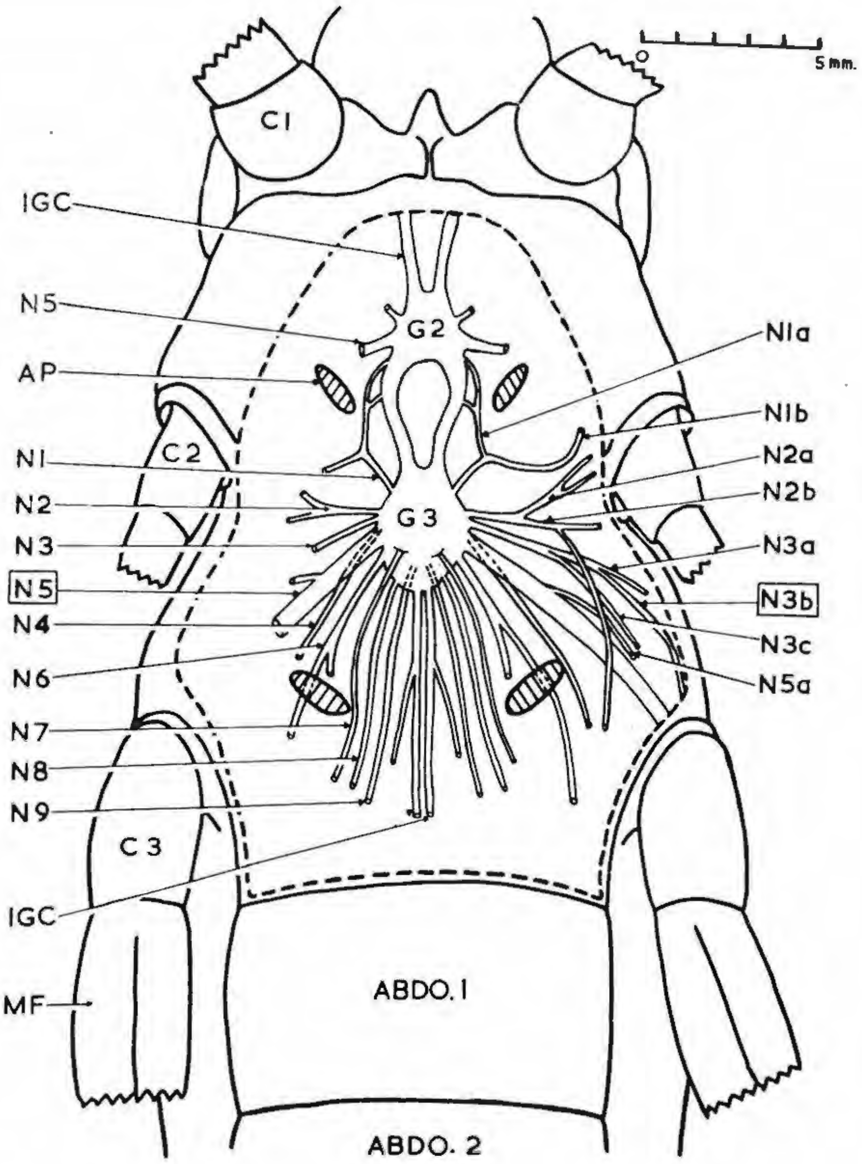


PLATE 1.1. A diagram of the opened thorax of Maphyteus leprosus, in ventral view, showing the ganglia and associated nerves. Nerve N5 and nerve N3b innervate the muscles of the metathoracic femur. N1 to N9 - Nerves 1 to 9; IGC - Interganglionic connectives; AP - Apodeme; MF - Metathoracic femur; G2, G3 - Meso-and metathoracic ganglia; C1, C2, C3 - Pro, meso- and metathoracic coxae; ABDO 1, ABDO 2 - First and second abdominal sternites. The cuticle of the sternites, within the limits of the broken line, has been removed.

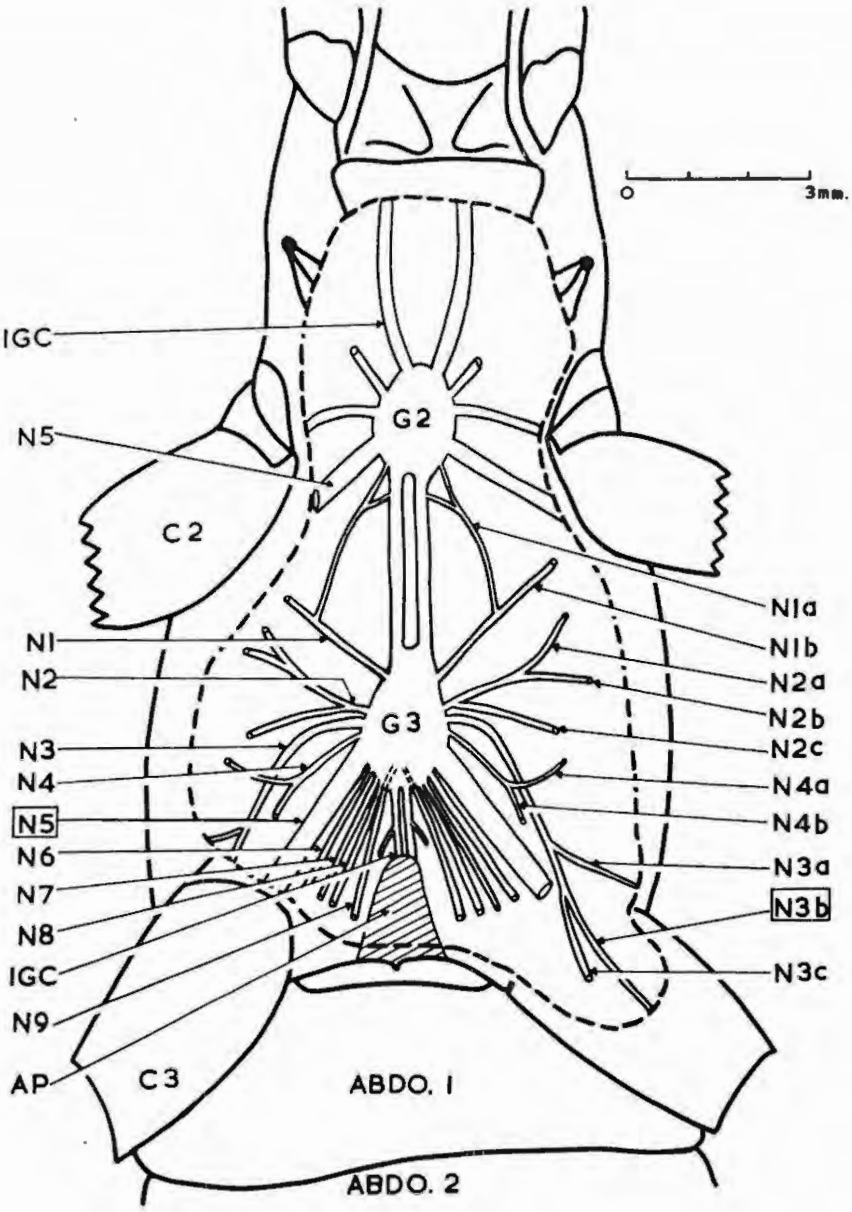


PLATE 1.2. A diagram of the opened thorax of Sphodromantis gastrica in ventral view, to show the ganglia and associated nerves. Nerve N5 and nerve N3b innervate the muscles of the metathoracic femur. Remainder of the legend as for Plate 1.1.

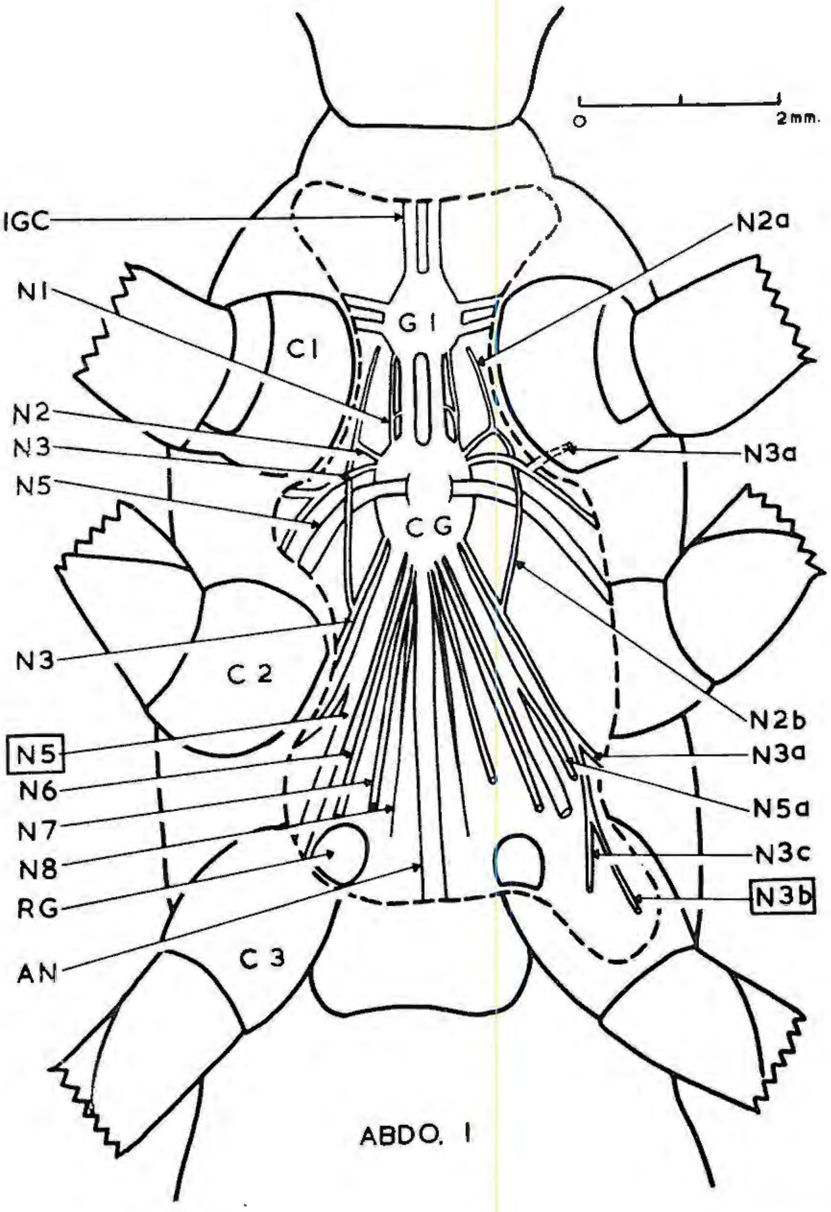


PLATE 1.3. A diagram of the opened thorax of *Ectrichodia crux* in ventral view, to show the ganglia and associated nerves. Nerve N5 and nerve N3b innervate the muscles of the metathoracic femur. RG - Repugnatorial gland; AN - Abdominal nerve. Remainder of the legend as for Plate 1.1.

Further, in the orthopteroids the more anterior nerve sends out branches before it enters the coxa while none arise from the more posterior nerve. This is not, however, true of Ectrichodia. Clearly conclusions drawn upon such flimsy evidence are not fully critical.

It might be expected that a definite conclusion could be reached by following the pathways of the two nerves and then deciding their homologies upon the basis of the structures which they innervate. This is not, however, possible as the two nerves join together in the trochanter, a single nerve trunk entering the femur in all cases.

The organization of the nerves within the femora call for brief comment. The arrangement of the nerves in the femora of Maphyteus and Periplaneta is essentially similar but in Sphodromantis and Ectrichodia there arises in the proximal end of the trochanter a slender branch from nerve 5 which is presumed to be sensory. In all four cases, however, the innervation and arrangement of the muscles and nerves within the femora closely fits the descriptions of other authors.

Since it is not possible to confirm the provisional identification of the two leg nerves by an anatomical study, a histological study was made of the nerves in the hope that they might show some characteristic organization of their axons.

The relevant nerves were dissected from the living animal, care being taken to handle the nerves only at one end which could later be discarded. Three fixatives were tried, Bouin, Alcoholic Bouin and Osmic Acid Potassium Dichromate. Of these Alcoholic Bouin proved to be completely satisfactory. Wigglesworth (1957)and Elftman (1957) have described techniques using as a fixative Osmic Acid buffered in Sodium Veronal Acetate with post-chromatization by potassium dichromate dissolved in mercuric chloride solution. Attempts to use this technique were not successful.

Considerable difficulty was experienced in developing suitable preparatory techniques for nerve sectioning. After numerous trials it was realized that the more robust nerves required relatively longer processing times than did the finer nerves. Thus for nerve 5 from *Maphyteus*, which is nearly 180 microns in diameter, suitable times are as follows:-

Alcoholic Bouin	3 hours.
80% Alcohol	3 changes during 4 hours.
90% Alcohol	3 changes during 12 hours.
Absolute Alcohol	Several changes during 12 hours.
Cedar wood Oil	6 hours.

while with a nerve of only 50 microns diameter the following schedule had to be used:-

Alcoholic Bouin	2 hours.
80% Alcohol	3 changes during 2 hours.
90% Alcohol	3 changes during 2 hours.
Absolute Alcohol	Several changes during 3 hours.
Cedar wood Oil	2 hours.

It was found convenient to stain the smaller nerves with light green in the clearing agent so as to prevent subsequent loss. After clearing, the material was embedded in paraffin wax in a vacuum oven. Sections were cut at 6 to 8 microns on a rotary microtome at a temperature of about 12°C. Most sections were cut with a standard microtome knife but Shick razor blades sharpened in the manner described by Wigglesworth (1959) were also used. A standard staining technique was adopted for all nerve sections as follows:-

Mordant in 3% Iron Alum at 50°C ...... 30 minutes.

Stain in Regaud's Haematoxylin
at 50°C .... 30 minutes.

Differentiate in $1\frac{1}{2}$% Iron Alum
at 20°C .... 2 to 3 minutes.

Clear in Xylene and mount in Canada Balsam.

Photomicrographs were made with a Zeiss Contaflex Super Single Lens Reflex 35 mm. camera fitted with a microscope attachment.

Efforts were made to cut serial sections of the legs in order to determine the axonal distribution to the femoral muscles in these animals. The toughness of the chitin prohibited the use of paraffin wax. Ester wax (Steedman 1947, 1957, 1960 a and b) and Chesterman and Leach (1956) was also tried unsuccessfully. Attempts to soften the chitin with mushroom extract (Carlisle 1960) were equally fruitless. Sections of the legs were, however, successfully cut using Wigglesworth (1959) Agar/Ester wax double embedding technique. Plates 1.4 and 1.5 are photomicrographs of sections of the femur of Sphodromantis and Ectrichodia to show the spatial arrangement of the muscles and nerves. However, even with this double embedding technique it proved impossible to obtain a complete series of sections and any attempt to reconstruct in this manner the ultimate pathways of the axons of nerves 3b and 5 had to be abandoned.

A series of photomicrographs of the leg nerves of Maphyteus, Periplaneta, Sphodromantis and Ectrichodia are shown in Plates 1.6 to 1.13. Each nerve is enclosed in a thick sheath (ns) which encloses not only tracheoles (tr) but very large numbers of nerve axons themselves enclosed within finer axon sheaths. Examination of the original sections shows the axons to fall into two major categories, large axons, which following Wigglesworth, are assumed to be motor axons, and fine axons which

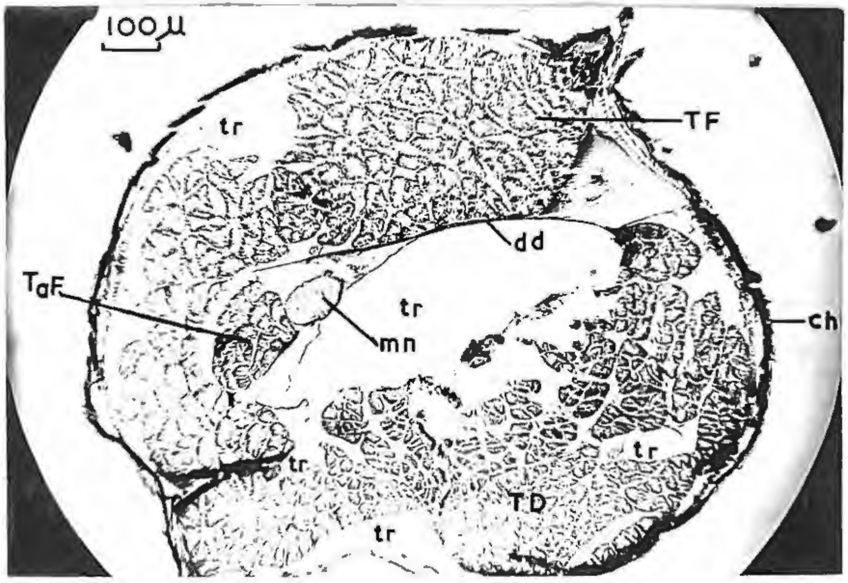


PLATE 1.4. Cross-section through the proximal portion of the metathoracic femur of Sphodromantis gastrica showing the spatial relationships of the muscles and nerves. TD - Flexor tibiae; TF - Extensor tibiae; dd - diaphragm dividing the extensor and flexor tibiae muscles; TaF - Tarsal flexor muscle; mn - the major nerve in the femur; tr - tracheae; ch - femoral exoskeleton.

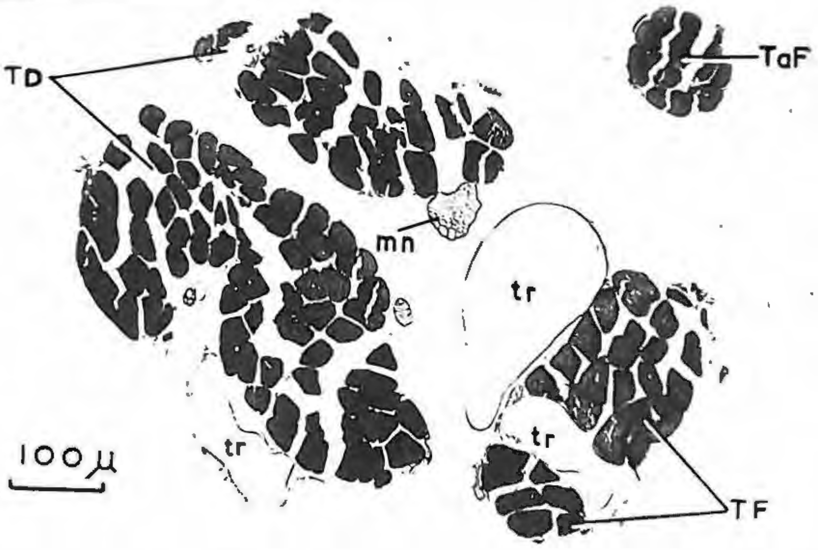


PLATE 1.5. Cross-section through the proximal portion of the metathoracic femur of Ectrichodia crux showing the spatial relationships of the muscles and nerves. TD - Flexor tibiae; TF - Extensor tibiae; TaF - Tarsal flexor muscle; mn - major nerve in the femur; tr - Tracheae. Exoskeleton removed to facilitate sectioning.

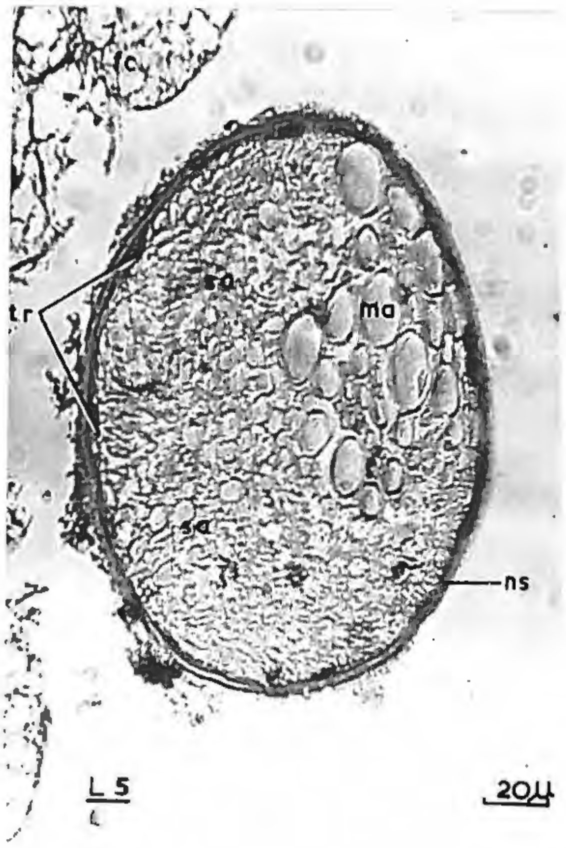


PLATE 1.6. A transverse section of Nerve N5 of *Maphyteus leprosus*, sectioned at 8 microns and stained in Regaud's Iron Haematoxylin. ma - motor area comprising large motor axons; sa - sensory area comprising small sensory axons; tr - tracheae; ns - nerve sheath (perilemma).

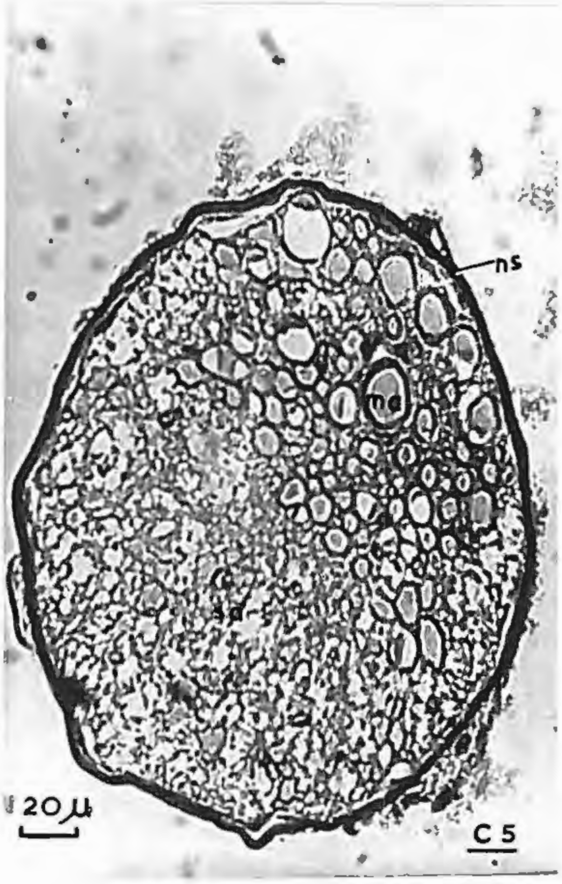


PLATE 1.7. A transverse section of Nerve N5 of Periplaneta americana, sectioned at 8 microns and stained in Regaud's Iron Haematoxylin. Remaining legend as for Plate 1.6.

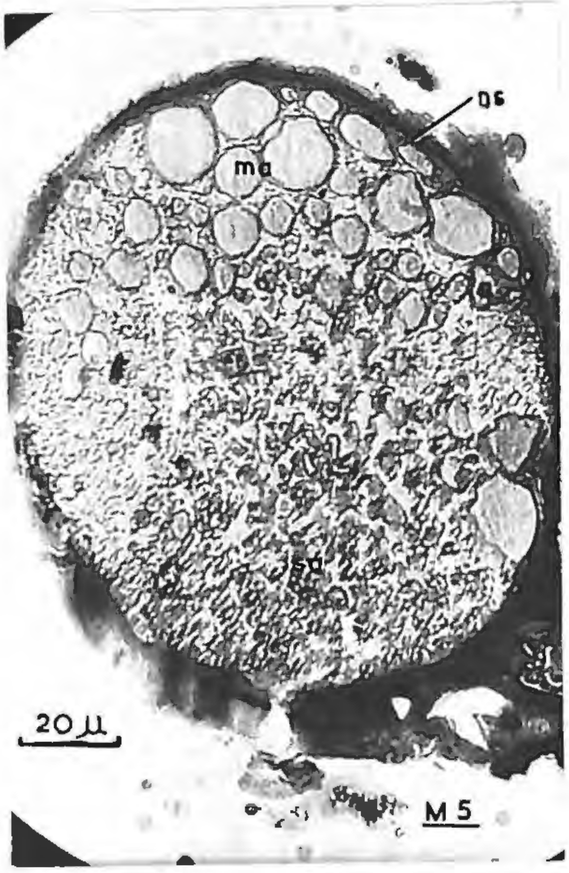


PLATE 1.8. A transverse section of Nerve N5 of Sphodromantis gastrica, sectioned at 8 microns and stained in Regaud's Iron Haematoxylin. Remaining legend as for Plate 1.6.

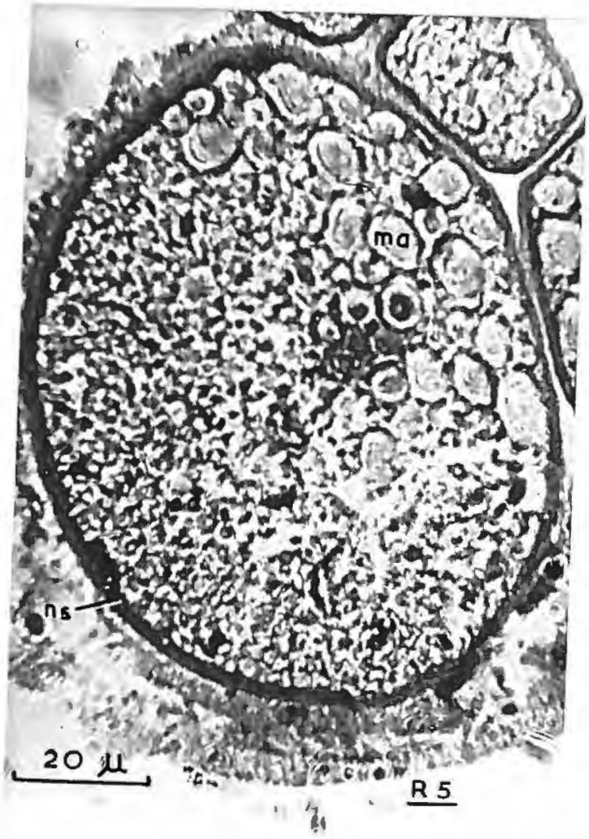


PLATE 1.9. A transverse section of Nerve N5 of *Ectrichodia crux* sectioned at 8 microns and stained in Regaud's Iron Haematoxylin. Remaining legend as for Plate 1.6.

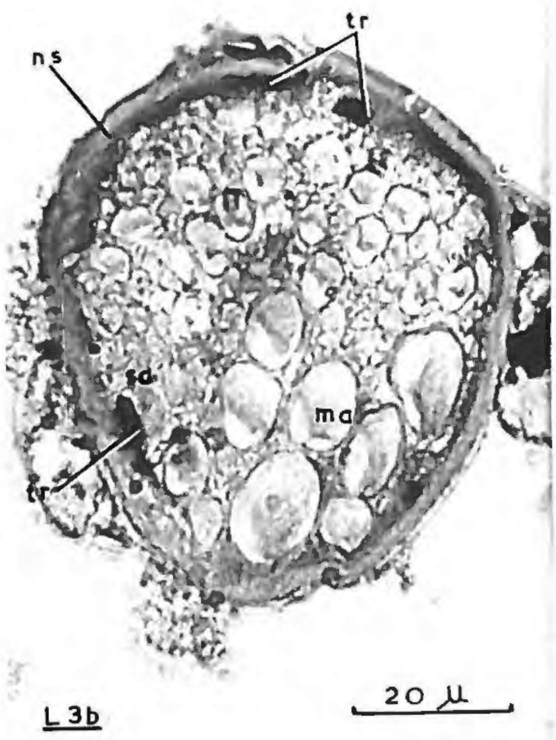


PLATE 1.10. A transverse section of Nerve N3b of Maphyteus leprosus, sectioned at 8 microns and stained in Regaud's Iron Haematoxylin. The remainder of the legend as for Plate 1.6.

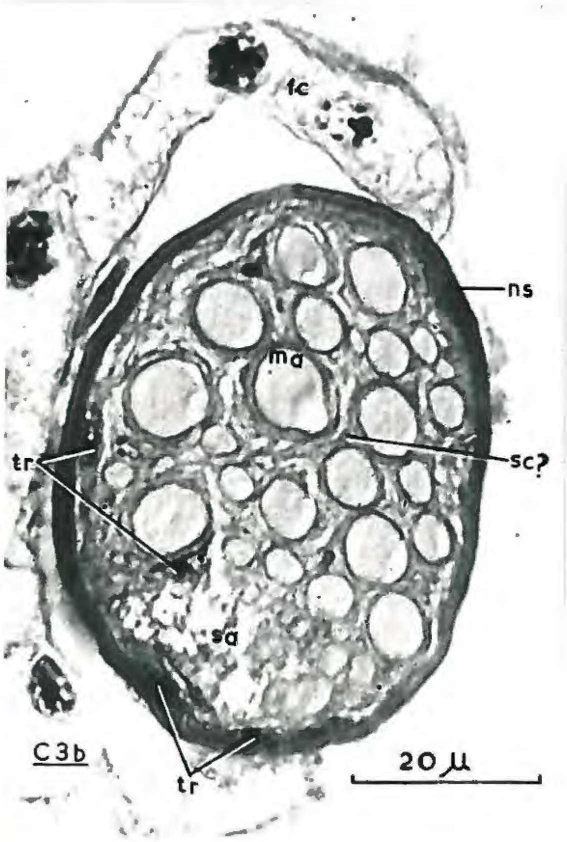


PLATE 1.11. A transverse section of Nerve N3b of *Periplaneta americana*, sectioned at 8 microns and stained in Regaud's Iron Haematoxylin. fc - cells of fatty tissues; sc - possible Schwann cell? The remainder of the legend as for Plate 1.6.

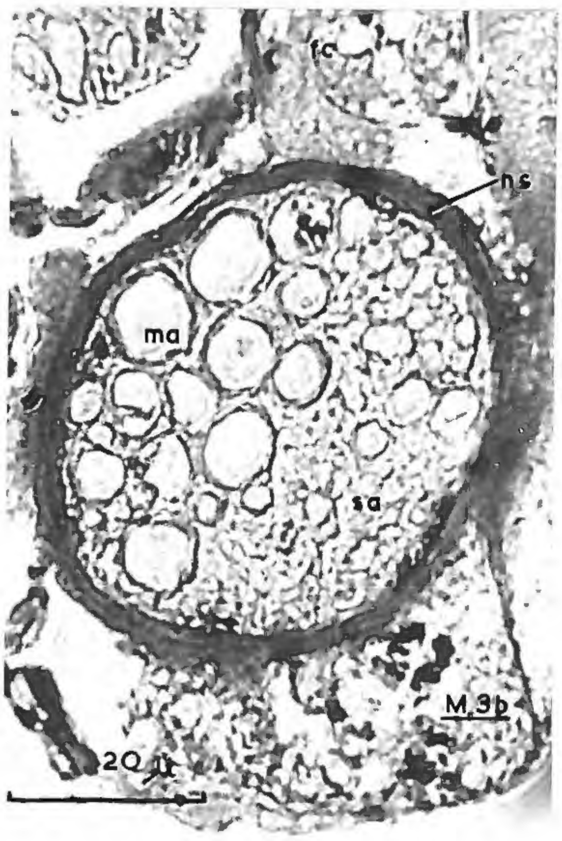


PLATE 1.12. A transverse section of Nerve N3b of *Sphodromantis gastrica*, sectioned at 8 microns and stained in Regaud's Iron Haematoxylin. fc - cells of fatty tissue. The remainder of the legend as for Plate 1.6.

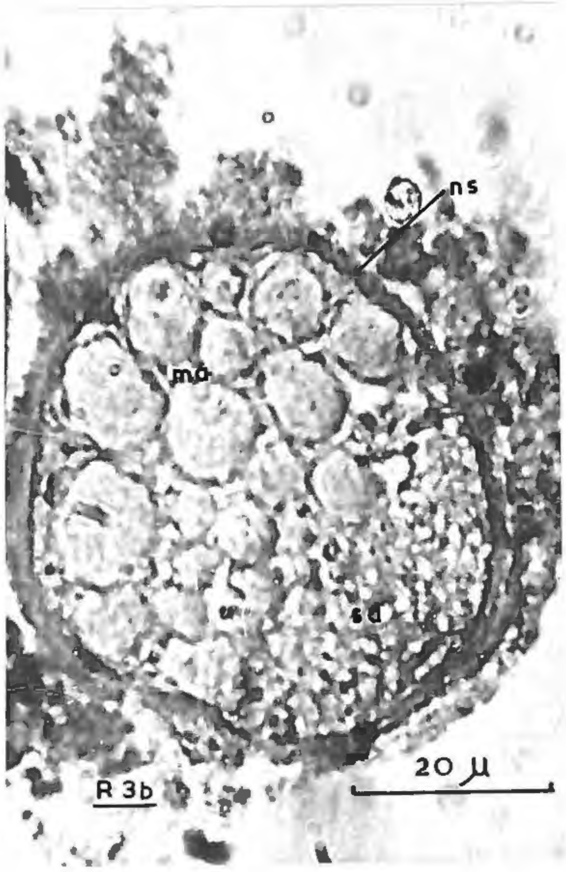


PLATE 1.13. A transverse section of Nerve N3b of *Ectrichodia crux*, sectioned at 8 microns and stained in Regaud's Iron Haematoxylin. The remainder of the legend as for Plate 1.6.

appear in the photomicrographs as an amorphous mass and are assumed to be sensory in function.

It is immediately apparent to cursory examination that in all cases the relative area occupied by the motor axons is far greater in the more anterior nerve, tentatively identified as nerve 3b, than in the more posterior nerve identified as nerve 5. A precise areal analysis is shown in Table 1.2.

TABLE 1.2.

Animal	Nerve	Total cross-sectional area in sq. μ.	Total area occupied by motor axons.	% area occupied by motor axons.	Total area occupied by sensory axons.	% area occupied by sensory axons.
Maphyteus	5	17473	9185	52.56	8289	47.44
Periplaneta	5	32419	21129	65.17	11290	34.83
Sphodromantis	5	9320	5253	52.36	4067	43.64
Ectrichodia	5	6244	2632	42.15	3612	57.85
				Av.% 54.06		Av.% 45.94
Maphyteus	3b	3284	2651	80.73	633	19.27
Periplaneta	3b	2284	1856	81.27	428	18.73
Sphodromantis	3b	1829	1407	76.92	422	23.08
Ectrichodia	3b	1954	1568	80.25	386	19.75
				Av.% 79.79		Av.% 20.21

The data show a further point of general interest. The total cross-sectional area of nerve 3b is of the same order in all four animals studied but there is a great difference in the cross-sectional areas of nerve 5. Some of this might be explained in terms of differences of size of the particular insects but even if allowance is made for this, nerve 5 of Periplaneta is still disproportinately large. One possible explanation lies in some correlation between axonal mass and activity. Neither Sphodromantis or Ectrichodia are active runners while Maphyteus is sluggish, relying on its repugnatorial glands and startle display for protection.

It is clear that these comparisons of the finer structure of the nerves support the provisional identification of nerves 3 and 5. There remains one final criterion. We have seen that the pathways of the various axons cannot be determined by gross anatomy. It is, however, possible to characterize the motor axons carried by these two nerves by direct electrical stimulation of their roots coupled with observations on the responses of the limb. Such an analysis has been undertaken and it is clear from Table 1.4 on page 42 that, except for the metathoracic extensor muscle of the grasshopper which is known to be exceptional, the results obtained, with the grasshopper and mantid at least, offer convincing support for the homologies that have been proposed.

Having thus ascertained, with some certainty, the homologies of the nerves supplying the femoral muscles, it remains to compare the thickness and structure of the nerve sheath (perilemma) in these four animals.

The thicknesses of the nerve sheaths surrounding nerves 5 and nerves 3b in Maphyteus, Periplaneta, Sphodromantis and Ectrichodia are summarised in Table 1.3.

TABLE 1.3.

Animal	Nerve	Nerve Major Axis	Nerve Sheath Thickness µ	Average Thickness µ
Maphyteus	5	180	2.08 - 2.40	2.24
Periplaneta	5	234	2.00 - 3.00	2.50
Sphodromantis	5	107	1.73 - 2.19	1.96
Ectrichodia	5	96	1.50 - 2.20	1.85
Maphyteus	3b	70	1.66 - 2.50	2.08
Periplaneta	3b	54	1.43 - 2.00	1.95
Sphodromantis	3b	65	1.60 - 2.30	1.71
Ectrichodia	3b	52	1.40 - 2.20	1.80

From this table it is clear that in all four animals the sheath surrounding nerve 3b is of approximately the same thickness. This immediately suggests that the idea proposed in the introduction, that correlated with a higher blood potassium, phytophagous and omnivorous animals might have thicker nerve sheath barriers than predatory forms, is erroneous. This conclusion receives strong support from the results obtained by Treherne in a recent series of papers (1960, 1961 a,b,c,d,e, and 1962) where he has demonstrated that, in the cockroach central nervous system, radioactive potassium and sodium ions are able to pass rapidly through the nerve sheath (perilemma) from the haemolymph, into an extracellular space between the nerve sheath and the nervous tissue itself. This is in direct opposition to the ideas of Hoyle and Twarog and Roeder. Treherne has shown that the concentration of sodium and potassium ions in the extracellular space beneath the perilemma is significantly higher than that in the haemolymph and that the chloride ion concentration is far lower in the former than in the latter. He suggests that the composition of this extracellular space may be governed by a Donnan membrane equilibrium. He postulates that when the perilemma is removed the resulting change in the ionic composition and especially the fall in concentration of sodium ion, permits a rapid depolarization of the nerve axons. Treherne has further demonstrated the presence of ion pumps which must lie within the nerve beneath the perilemma.

Treherne's studies are as yet incomplete and his experiments have mostly been made with the nervous tissues bathed in salines of low potassium concentration, lower than those normally found in cockroach blood and far lower than those reported for phytophagous insects. One is led to conclude that the "deep" ion pumps are the effective barriers which protect the individual nerve axons from potassium block, but this is as yet unproven.

What is, however, clear is that the perilemma itself is not a diffusion barrier and it is therefore not to be expected that its development will reflect the ionic composition of the blood.

Indeed the more remarkable aspect of this sheath is its constancy of thickness around nerves of comparable size. Apart from its role as a barrier to large anionic molecules which allow the establishment of a Donnan equilibrium, it seems likely that the sheath serves a purely mechanical function. Twarog and Roeder (1956) have described an "explosive bursting out" of nerve substance from the nerves of Periplaneta when the sheath is pierced. This is probably due to the fact that the osmotic pressure within the perilemma is greater than that of the haemolymph and the perilemma is required to meet the mechanical pressure arising from the difference in osmotic pressure. If then the sheath is a mechanical necessity, it is possible that the larger bundles of axons require a more robust wrapping. The cellular perineurium of the perilemma does not extend into the nerves and the differences in sheath thickness recorded here relate only to variations in thickness of the fibrous neural lamella, that is they reflect mechanical rather than metabolic properties of the nerve sheath. It is thus very likely that these differences in thickness of the neural lamella correlate with mechanical factors and have no relation to protection of the nerve axons from the ions of the haemolymph.

Treherne's studies upon ion fluxes in the nervous system of Periplaneta make it clear that the perilemma is freely permeable to ions and inulin while Wigglesworth (1960) has shown that the neural lamella does not exclude trypan blue. It seems unlikely therefore that the perilemma will constitute an effective barrier to the entry of drugs. The deeper barrier whose presence is suggested by Treherne's studies may possibly protect the axons from drugs.

Clearly the properties of these deeper barriers can as yet only be approached indirectly and a preliminary investigation of their properties has been made by comparing their efficacy in protecting the axons against varying external potassium ion concentrations in the different insects being studied.

It will be shown in the following pages that the nerves of the entomophagous Sphodromantis are more susceptible to high external potassium ion concentrations than those of the phytophagous Maphyteus. This observation is open to two interpretations and further preliminary studies of drug action on these animals, however, favour one interpretation rather than the other.

APPARATUS AND TECHNIQUES.

The arrangement used in the present experiments follows the procedure of other workers (Hoyle 1953, Ripley 1954) and calls for almost no comment. As has been emphasised elsewhere care must be taken to avoid damaging the salivary glands. In the reduviid these are extremely large and overlie the nervous structures in the thorax. Further nerves in Ectrichodia are not found superficially just beneath the ventral sclerites but run through a mass of tissue and beneath the coxal cavities of the meso- and metathoracic limbs. As a result such preparations are difficult and unsatisfactory: the main study of the neuromuscular physiology of an entomophagous form has therefore been upon the mantid.

The operative procedure usually takes 20 to 30 minutes, the tissues remaining bathed in the animal's haemolymph during this time. Initial records of both mechanical and electrical responses of the muscle were made in the haemolymph, but subsequently the preparation was moistened with saline or the haemolymph completely replaced by artificial solutions. A wide variety of salines have been developed for use with insects (Roeder and

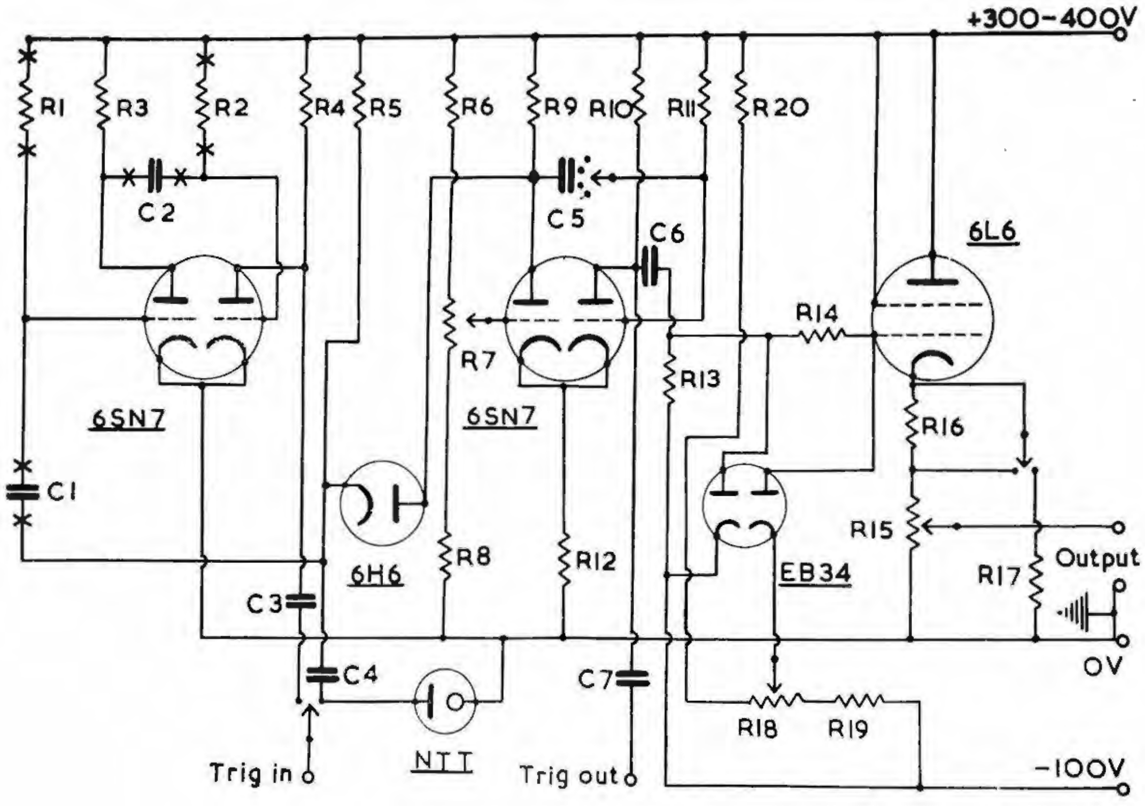
Roeder 1939, Griffiths and Tauber 1943, Hoyle 1953, Barsa 1954, Becht, Hoyle and Usherwood 1960, Yamasaki and Narahashi 1959 and 1960, Vereshtchagin et al. 1961.) Of these Hoyle's (1953) standard saline was used without addition of dextrose.

Again following normal practice the nerve trunks were stimulated by square waves. These were supplied from a stimulator built to Ead's (1951) design (Plate 1.14), a voltage regulator and stabilizer circuit following the design of Attree (1955, quoted in Donaldson 1958) (Plate 1.15), being included between the power pack (Plate 1.16) and the stimulator. Ead's stimulator employs two controlled multivibrator stages as does that of Bernstein (1950). Experience has shown that the latter, although simpler in design, is unstable if continuously variable controls are used and with preset values it offers a far smaller range of repetition frequencies.

Mechanical recordings were made on a Palmer Electrical Kymograph using either frontal or gimbal levers with Locusta and Maphyteus. The laboratory temperature was raised to approximately 24°C and the relative humidity raised to 70 - 80% before the start of any experiment.

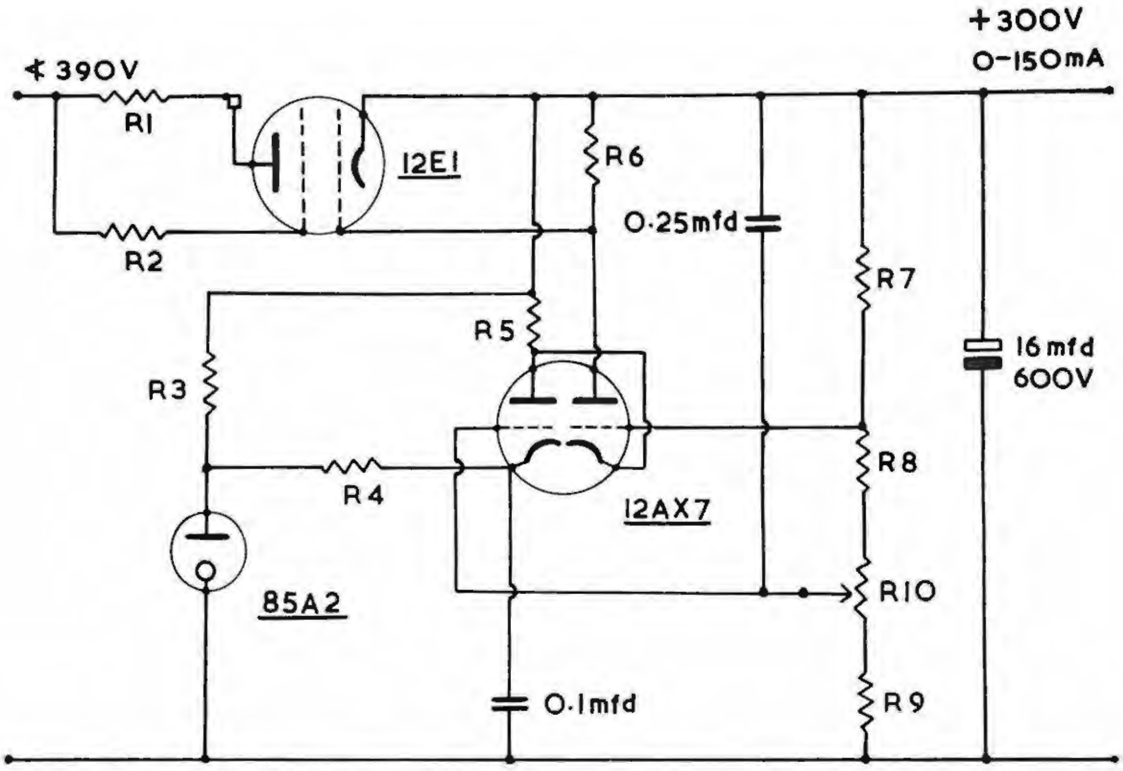
RESULTS.

A series of experiments were conducted in which the responses of the tibial extensor and flexor muscles of Maphyteus and Sphodromantis were examined using Hoyle's saline. These experiments were conducted in order to gain a knowledge of the innervation patterns of the mantid and to establish whether the innervation patterns in the metathoracic limb of Maphyteus, which is not markedly specialized for jumping, is the same as those described by Hoyle (1955a,1957) for the powerful, jumping, metathoracic limbs of Schistocerca and Locusta. Hoyle has also described the innervation patterns in the pro- and mesothoracic limbs of Schisto-



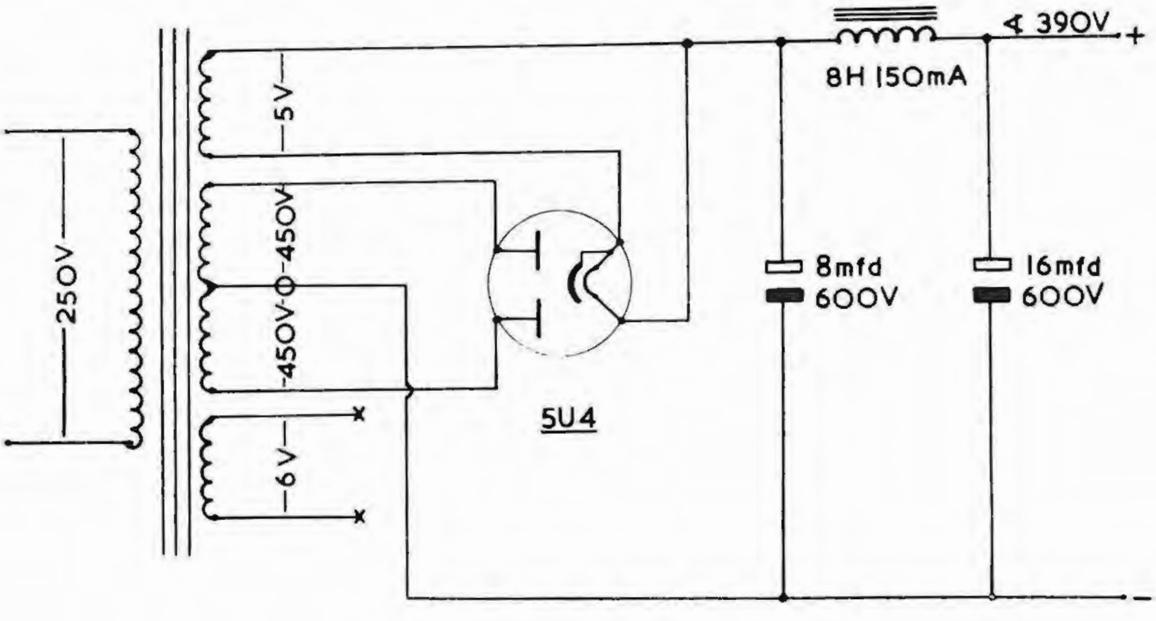
EAD'S STIMULATOR

PLATE 1.14. Circuit diagram of Ead's (1951) stimulator. The values of the fixed resistances are as follows:- R3, R4 - 47 k; R5 - 1M; R6 - 250 k; R8 - 22 k; R9, R10 - 20 k; R11 - 5M; R12 - 10 k; R13 - 1M; R14 - 47 k; R16 - 18 k; R17 - 2.2 k; R19 - 100 k; R20 - 200 k. There are three potentiometers, R7 - 25 k; R15 - 2.2 k; and R18 - 100 k. The switched values of the resistances R1 are 20M and 10M, and those of R2 are 20M, 10M, 4.7M, 3M, 1.5M, and 1M. The values of the condensers are:- C3 - 0.1μF; C4 - 0.0001μF and C6, C7 - 1.0μF. The switched values of condenser C1 are 0.05μF, 0.001μF, and 0.0001μF; and those of C2 are 2μF, 0.05μF and 0.001μF. (A table of switched RC values for each stimulus frequency is given by Ead (1951) and Donaldson (1958)). The duration of the stimulus output is controlled largely by the bank of four condensers C5 whose values are 0.0001μF, 0.001μF, 0.01μF and 0.1μF.



VOLTAGE REGULATOR & STABILIZER

PLATE 1.15. Circuit diagram of Attree's (1955) voltage regulator and stabilizer. The values of the fixed resistances are as follows:- R1 - 10Ω; R2 - 47Ω; R3 - 47k; R4 - 100Ω; R5 - 220k; R6 - 2.2M; R7 - 33k; R8 - 15k; R9 - 18k. There is one 5k potentiometer R10.



POWER PACK

PLATE 1.16. Circuit diagram of the power pack.

cerca and Locusta and the innervation of the tibial muscles in these two segments of Maphyteus and Sphodromantis was investigated to establish whether Hoyle's results are generally applicable.

Experiments were then carried out to study the effects of changes in ionic composition of the "haemolymph" on the mechanical responses of these muscles.

The discussion of the results may conveniently be divided into four sections:-

1. The responses of the tibial extensor and flexor muscles to nervous stimulation.
2. The effects of changes in the ionic composition of the saline on the mechanical responses of the muscles.
3. The effects of changes in the ionic composition of the saline on the threshold of the nerves.
4. The action of drugs on phytophagous and predatory forms.

1. THE RESPONSES OF THE TIBIAL EXTENSOR AND FLEXOR MUSCLES TO NERVOUS STIMULATION.

(A) RESPONSES OF MAPHYTEUS METATHORACIC TIBIAL MUSCLES.

The following technique was adopted in all these experiments. Firstly the apodeme of the muscle antagonistic to that about to be tested was cut, thus severing the muscle from the tibia. Next the thorax was opened and the nerve innervating the muscle was ligatured and hooked up to the stimulating electrodes. The rest of the nerves coming from the ganglion were cut. In all experiments the thresholds for response were measured at a stimulation frequency of 35 stimuli per second with a pulse duration of approximately 1 msec.

(i) Responses of the extensor tibiae with stimulation of nerve 5. (Preparation a).

The extensor tibia muscle of the grasshopper shows a typical fast tetanic response at frequencies above 20 stimuli per second (Trace 1.1). The threshold for this response is ca. 0.34 volts. This value is dependent to some extent on the size of the animal but the results quoted all refer to large females and the thresholds were found to be very consistent in these cases. At high stimulation frequencies (20 per second or more) there is no recruitment of muscle fibres with increases in stimulation intensity. The contraction is all-or-none.

The muscle responds with discrete twitches to low stimulation frequencies from 1 to 3.0 per second. At a stimulus frequency of 6 per second the twitches summate into a clonic contraction. At 20 stimuli per second the muscle responds with a smooth tetanus (Traces 1.2 and 1.3). These results are in accordance with Hoyle's findings (1955a and b, 1957).

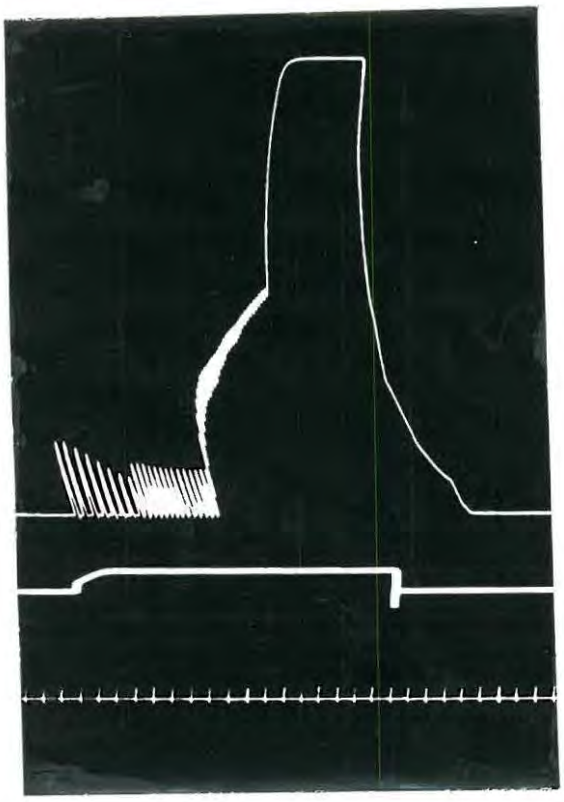
In some experiments, the fast response shown by this muscle appeared to be inhibited by stimulation at certain intensities; this effect was, however, eliminated by the insertion of a 0.5 microfarad condenser in the output lead of the stimulator as recommended by Hoyle (1957).

A slow response could not be evoked from this muscle with stimulation of nerve 5 at any stimulation frequency, duration or intensity.

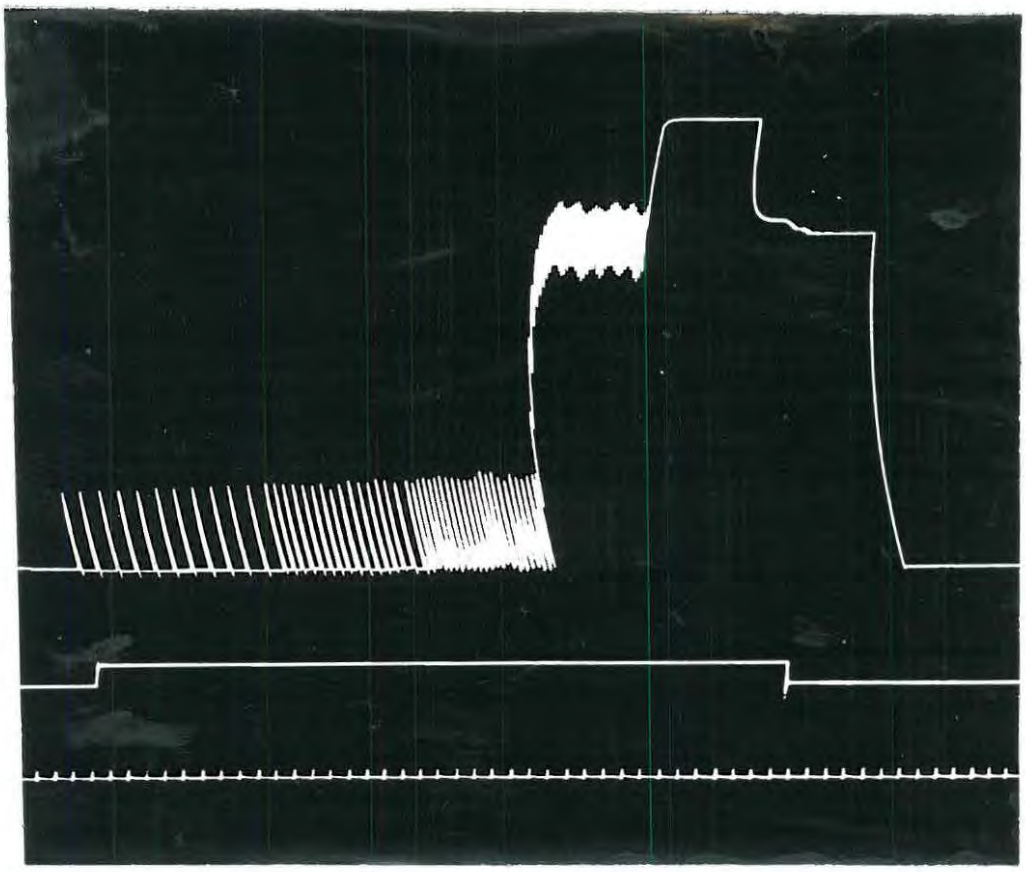
(ii) Responses of the extensor tibiae with stimulation of nerve 3. (Preparation b).

Hoyle reports (1955a, 1957) that the extensor tibiae of the grasshoppers Schistocerca and Locusta receives two "slow" axons via nerve 3. These he names S^1 and S^2. Stimulation of the latter produces no mechanical response in the muscle but stimulation of S^1 results in a "slow" contraction of the muscle. The speed of extension during the slow response varies considerably, depending on the frequency of stimulation, from a very slow ex-

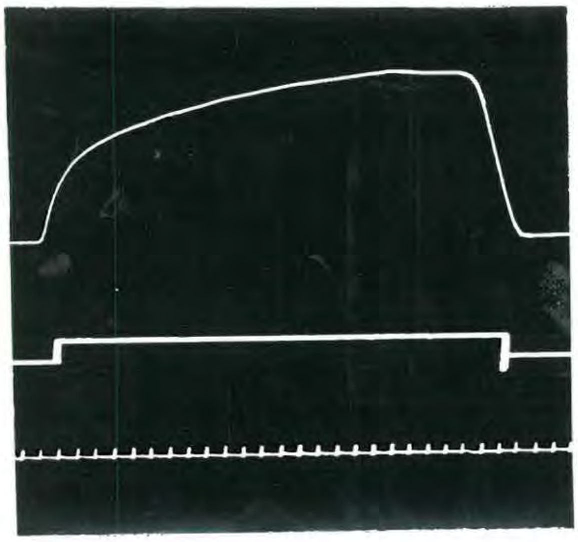
TRACE 1.1. "Fast" tetanic response of Maphyteus metathoracic extensor tibiae muscle with stimulation of nerve 5. (Preparation a). Frequency 20 per second. Time trace: 1 sec.



TRACE 1.2. Responses of Maphyteus metathoracic extensor tibiae muscle, with stimulation of nerve 5, (Preparation a), to increases in stimulation frequency. Frequency increments 2, 3.2, 6 and 20 per second. Time trace: 1 sec.



TRACE 1.3. Frequency responses of Maphyteus metathoracic extensor tibiae muscle with stimulation of nerve 5. (Preparation a). Frequency increments 1, 2, 3.2, 6, and 20 per second. Time trace 1 sec. (Note the relaxation delay after cessation of the stimulus which was apparently due to some defect in the mechanical recording apparatus.)



TRACE 1.4. "Slow" response of Maphyteus metathoracic extensor tibiae muscle with stimulation of nerve 3. (Preparation b). Stimulation frequency 20 per second. Time trace: 1 sec.

tension taking several seconds to develop, to a very rapid extension. This rapid extension is noticeably slower than the fast extension which results from stimulation of the fast axon in nerve 5. The fast fibre in nerve 5 is utilized when the animal jumps.

In Maphyteus stimulation of nerve 3 evokes from the extensor muscle responses which differ from those shown when nerve 5 is stimulated. At a frequency of 20 shocks per second and at a critical threshold of ca. 0.40 volts depending on the animal, a slow response can be evoked which takes several seconds to develop fully (Trace 1.4). At a higher frequency of 35 shocks per second there is a much more rapid contraction from the muscle which is broadly similar in appearance to the fast response in preparation (a) yet not as rapid as the latter.

Below 20 shocks per second the muscle responds with very small discrete twitches which were too weak to be recorded while the tibia was attached to the writing lever. Hoyle reports similar "minute" twitches with this preparation but only at a frequency below 15 shocks per second, and only with exceptionally large animals.

(iii) Responses of the flexor tibiae with stimulation of nerve 5. (Preparation c).

The flexor tibiae muscle of Maphyteus responds to high frequency stimulation (35 shocks per second and above) of nerve 5 with a normal fast tetanic contraction. The threshold for the response is ca. 0.24 volts. At threshold intensity and low frequencies of stimulation the muscle responds with rather small discrete twitches. The muscle shows a clonic response at 20 stimuli per second, and a tetanic response at 35 stimuli per second (Trace 1.5). In preparation (c) there is some evidence to suggest that the flexor muscle is supplied with several fast axons (i.e. it is polyaxonic) as reported by Hoyle. If the writing lever is disconnected from the tibia and the flexor muscle stimulated into tetanus at threshold intensity, subsequent increments in stimulation intensity or frequency, result in the muscle going into tighter and tighter tetanic contraction. The resultant movements of the tibia are very slight (because the limb is almost maximally flexed) but are indicative of at least three contraction levels.

Hoyle has never recorded a slow response from the flexor tibiae muscles of Locusta or Schistocerca. An apparent slow response has, however, been recorded from the flexor tibiae of Maphyteus, when nerve 5 is stimulated at a frequency of 20 shocks per second, provided the stimulation intensity is twice threshold for the fast response (Trace 1.6). This would be in accordance with the results of Wood (1958) who presents rather unconvincing mechanical records showing the slow response from the prothoracic flexor of Carausius; and of Wilson (1954) who claims to have recorded characteristic slow muscle action potentials from the flexor tibiae muscle of the cockroach. There is good reason to believe, however, that in the case of the metathoracic flexor of Maphyteus, the slow response is the result of a recording lever which was too heavy for the muscle. Subsequent recording with a lighter lever failed to show any indication of this slow response.

(iv) Responses of the flexor tibiae with stimulation of nerve 3. (Preparation d).

The flexor tibiae of the grasshopper does not respond to stimulation of nerve 3 at any frequency, intensity or duration. In some cases, at exceptionally high stimulation intensities, (more than 10 times threshold for the normal response), the muscle was seen to respond. In these cases electrotonic spread can account for these responses. The current spread must stimulate either nerve 5 or the muscle directly.

(B) RESPONSES OF SPHODROMANTIS METATHORACIC TIBIAL MUSCLES.

The innervation patterns and responses of the metathoracic tibial muscles of the mantid differ in several respects from the condition described for the grasshopper metathoracic limb muscles. The greatest difficulty was experienced in obtaining mechanical recordings from the tibia of Sphodromantis. In fact it was impossible to make recordings from the tibia with even the lightest of levers. The extremely thin tibial cuticle buckled as a result of contraction of the tibial muscles. Ripley (1949) has had success with mechanical recordings from the tarsus of a grasshopper, using a straw lever but even a lever of this sort proved to be too heavy. A light balsa wood lever was devised which would record from the tibia but this had such an adverse effect on the mechanical response of the muscle that it also was eventually rejected. For want of anything better a close visual observation was kept on the tibia during experiments and the responses of the muscles recorded diagrammatically.

(i) Responses of the extensor tibiae with stimulation of nerve 5. (Preparation e).

The extensor tibiae of the mantid does not respond to stimulation of nerve 5 at any frequency, intensity or duration. Nerve 5 innervates several muscles in the coxa, trochanter and tarsus but not the muscles in the femur.

(ii) Responses of the extensor tibiae with stimulation of nerve 3. (Preparation f).

Stimulation of nerve 3 at frequencies of 35 stimuli per second or more results in a fast tetanic contraction from the tibial extensor muscle. The threshold (ca. 0.09 volts) for this response is lower than those for any of the grasshopper preparations a, b and c.

At frequencies below 35 stimuli per second the muscle responds with discrete twitches. At a stimulation frequency of 5 shocks per second the muscle twitches will summate with increasing intensity of stimulation, while at higher intensities still they fuse into a complete tetanus.

With high frequency stimulations (35 per second) and with gradually increasing intensities, there is a clear recruitment of muscle fibres. Four obvious levels of tension are developed. With a constant stimulation intensity (held just above threshold level) a similar recruitment of muscle fibres may be induced by stimulation frequency increments. Here again there are four distinct tension levels.

With low frequency stimulation (3.0 per second) the twitches summate to a definite level and the muscle remains in clonic contraction at that level. An increase in stimulation frequency to 6 shocks per second results in a fresh summation of the twitches until a new clonus level is achieved. With augmentation of stimulation frequencies to 20 or 35 shocks per second, the summation of the twitches is so rapid that the new clonus level is attained almost immediately. It is also evident that the tetanus fusion frequency of the extensor muscle in the mantid is far higher than that of the grasshopper. The latter shows smooth tetanus at 20 stimuli per second while the mantid extensor tibiae still twitches slightly at 35 stimuli per second. Completely smooth tetanus is only achieved at higher frequencies with the mantid. This difference in fusion frequency is also manifest in the tibial flexor muscles of the metathorax, and the tibial flexor and extensor muscles of the pro- and mesothorax.

Stimulation of nerve 3 at intensities just below the threshold for the quick response (ca. 0.08 volts) elicits a definite slow contraction from the extensor muscle. The slow contraction takes from 2 - 5 seconds to develop to full tension. Although the response recorded from this muscle, under these conditions, has every appearance of a genuine slow contraction, in some cases it was evident that the slow contraction was made up of very minute summating twitches. It seems possible that the slow response is an artifact brought about by stimulation of the fast axon just below its threshold intensity. This probably also applies to the extensor muscles in the pro- and mesothoracic segments. However, it is important to note that Pringle (1939) has described a fast and a slow axon in nerve 3b of Periplaneta which supplies the metathoracic extensor tibiae muscle.

It may thus be concluded that the extensor tibiae of the mantid, innervated by nerve 3, is supplied with at least four motor axons (shown by the four distinct recruitment levels), which produce a fast response, and possibly one axon which evokes the slow response.

(iii) Responses of the flexor tibiae with stimulation of nerve 5. (Preparation g).

The flexor tibiae muscle of the mantid responds with a fast contraction to stimulation of nerve 5 at high frequencies (35 shocks per second). The threshold intensity for this response is ca. 0.18 volts and is higher than the threshold for the fast response in the extensor muscle (preparation f). The mechanical characteristics of the fast response of the flexor muscle are very similar to those described for preparation (f). In common with preparation (f) the flexor muscle only attains smooth tetanus at stimulation frequencies of above 35 shocks per second. There is twitch summation at low stimulation frequencies and the muscle attains different levels of contraction with changes in stimulation frequency, intensity and duration. These results are in agreement with Hoyle who reports that the flexor tibiae muscles of the Orthoptera (sensu stricto) are polyaxonic.

A slow response has never been recorded from preparation (g) at any stimulation frequency, intensity or duration.

(iv) Responses of the flexor tibiae with stimulation of nerve 3. (Preparation h).

Stimulation of nerve 3 does not produce any mechanical response from the flexor tibiae muscle of the mantid. This has been confirmed in several preparations.

(C) RESPONSES OF MAPHYTEUS AND SPHODROMANTIS MESOTHORACIC AND PROTHORACIC TIBIAL MUSCLES.

It is clear from the preceding that the innervation patterns and mechanical responses of the metathoracic tibial muscles of the grasshopper and mantid are different. This point will be elaborated in the discussion.

Hoyle (1957) has reported that the innervation patterns to the extensor and flexor muscles in *Locusta*, in meso- and prothoracic segments, are identical but differ from those of the metathorax. The pro- and mesothoracic tibial extensor muscles each receive a single fast axon through nerve 3b while each is innervated by a slow axon running in nerve 5. The flexor tibialis muscles of the pro- and mesothoracic segments are innervated by at least four fast axons which run in nerve 5. This has been confirmed in *Locusta* and *Schistocerca* by Hoyle (1957). The pro- and mesothoracic flexor muscles of *Locusta* and *Schistocerca* are not innervated by any slow axons.

It seemed desirable to try to confirm these observations and extend them to a comparison of the innervation patterns of the pro- and mesothoracic segments in *Maphyteus* and *Sphodromantis*. The results obtained from this investigation are presented in Table 1.4.

Three of the responses recorded in Table 1.4 require further

TABLE 1.4.

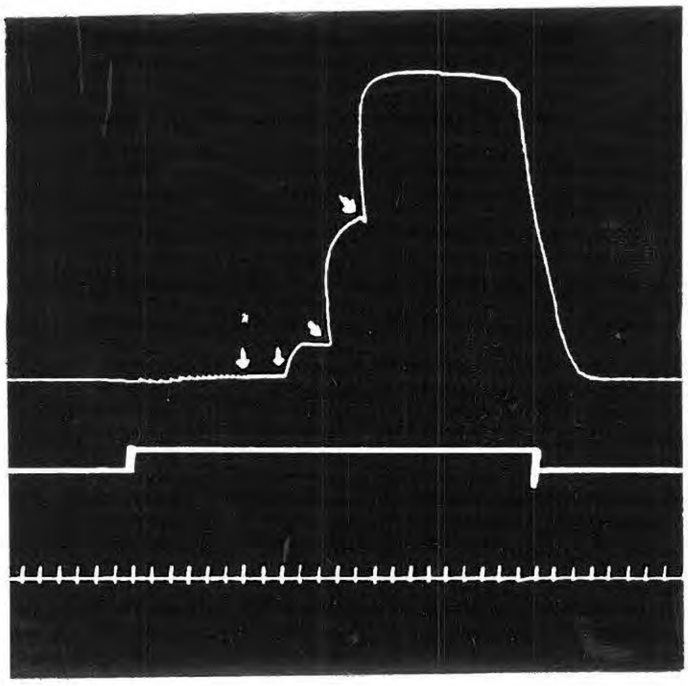
ANIMAL	SEGMENT	MUSCLE	NERVE	RESPONSE	THRESHOLD (VOLTS)
Maphyteus	Metathoracic	Extensor	5	Fast	0.34
Maphyteus	Metathoracic	Flexor	5	Fast	0.24
Maphyteus	Metathoracic	Extensor	3	Slow	0.40
Maphyteus	Metathoracic	Flexor	3	No response	-
Sphodromantis	Metathoracic	Extensor	5	No response	-
Sphodromantis	Metathoracic	Flexor	5	Fast	0.18
Sphodromantis	Metathoracic	Extensor	3	Fast+(Slow?)	0.09
Sphodromantis	Metathoracic	Flexor	3	No response	-
Maphyteus	Mesothoracic	Extensor	5	No response	-
Maphyteus	Mesothoracic	Flexor	5	Fast	0.25
Maphyteus	Mesothoracic	Extensor	3	Fast	0.21
Maphyteus	Mesothoracic	Flexor	3	No response	-
Sphodromantis	Mesothoracic	Extensor	5	No response	-
Sphodromantis	Mesothoracic	Flexor	5	Fast	0.17
Sphodromantis	Mesothoracic	Extensor	3	Fast+(Slow?)	0.13
Sphodromantis	Mesothoracic	Flexor	3	No response	-
Maphyteus	Prothoracic	Extensor	5	No response	-
Maphyteus	Prothoracic	Flexor	5	Fast	0.26
Maphyteus	Prothoracic	Extensor	3	Fast	0.22
Maphyteus	Prothoracic	Flexor	3	No response	-
Sphodromantis	Prothoracic	Extensor	5	No response	-
Sphodromantis	Prothoracic	Flexor	5	Fast	0.28
Sphodromantis	Prothoracic	Extensor	3	Fast+(Slow?)	0.24
Sphodromantis	Prothoracic	Flexor	3	No response	-

explanation.

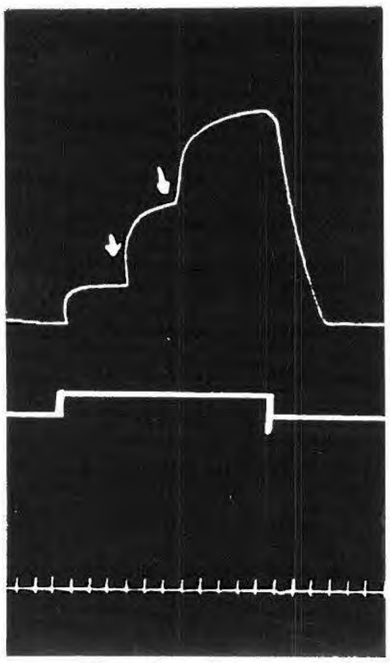
(i) In the case of the grasshopper there is some variability associated with the slow response of the mesothoracic extensor muscle with stimulation of nerve 3. In the majority of preparations there was a definite slow response, but in some a slow response could not be evoked with any stimulus. In addition, in those animals in which a slow response was recorded, the critical stimulus intensity lay within very fine limits and was usually just below the intensity required to evoke a fast response. Thus it seems that only some of the shocks reaching the nerve are stimulating the axon at threshold for the fast response and this "counting down" is responsible for the appearance of an apparent slow response. Hoyle does not record a slow axon in nerve 3 in Locusta or Schistocerca and because of the difficulty and inconsistency in obtaining a slow response from the Maphyteus pro- and mesothoracic extensor tibiae preparation, it is felt that these results need further confirmation and should be regarded with suspicion.

(ii) The responses of the grasshopper mesothoracic flexor tibiae with stimulation of nerve 5 call for some comment. The fast response from this muscle is characterized by a definite peripheral recruitment of muscle fibres with changes in the stimulation frequency, intensity and duration. This brings to mind the very similar responses recorded from the mantid preparation (f). The recruitment of muscle fibres in the mesothoracic flexor tibiae of the grasshopper with alteration of frequency, duration and intensity is shown clearly in Traces 1.7, 1.8 and 1.9. These results suggest the presence of atleast four fast axons running in nerve 5 which innervate the mesothoracic flexor and are consistent with the results of other workers.

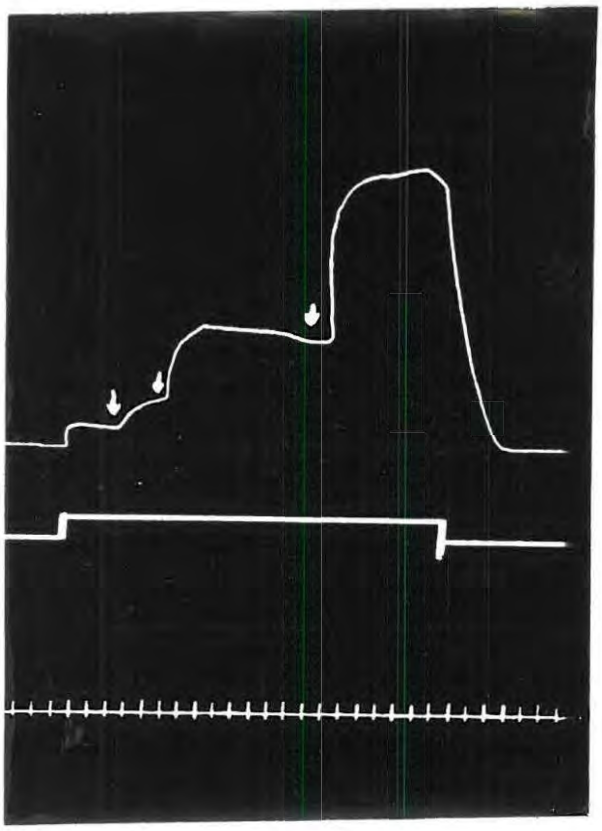
(iii) Hoyle (1957) reports that the pro- and mesothoracic extensor tibiae muscles of Locusta and Schistocerca are each



TRACE 1.7. Effects of frequency increases (indicated by small arrows) on the fast response shown by the Maphyteus mesothoracic flexor tibiae muscle with stimulation of nerve 5. Frequency increments 2, 3.2, 6, 20, 35 and 120 per second. Time trace: 1 sec.



TRACE 1.8. Effects of duration increases (indicated by small arrows) on the fast response shown by the mesothoracic flexor tibiae muscle of Maphyteus, with stimulation of nerve 5. Time trace: 1 sec.



TRACE 1.9. Effects of intensity increases (indicated by small arrows) on the fast response shown by the mesothoracic flexor tibiae muscle of Maphyteus, with stimulation of nerve 5. Time trace: 1 sec.

innervated by a slow fibre running in nerve 5. The pro- and mesothoracic extensor tibiae muscles of Maphyteus and Sphodromantis were never seen to respond mechanically in any way to stimulation of nerve 5.

Table 1.4 shows that in general both the mechanical responses and the innervation patterns of the mantid and the grasshopper are identical except for the grasshopper metathoracic extensor tibial muscles which do not conform with the rest. In this case the extensor tibiae receives an extra fast fibre via nerve 5. Evidently this extra innervation has to do with the very large size of the metathoracic tibial muscles and the specialization of the limb as a saltatorial organ. There is no evidence to support the supposition that because Maphyteus is a very weak jumper, the innervation patterns of the metathoracic limbs differ in any way from the condition reported by Hoyle for the very powerfully saltatorial limbs of Locusta or Schistocerca. The innervation patterns to the metathoracic limbs of three other grasshoppers, characterized by varying degrees of saltatorial adaptation, namely Zonocerus elegans, Dictyophorus spumans and Acanthacris ruficornis have also been studied in the course of this investigation and found to conform exactly to each other and to the other three species, namely Maphyteus, Schistocerca and Locusta.

A study of the threshold intensities, for the fast muscle response, recorded in Table 1.4 reveals some interesting data. There is a direct correlation between the size of the muscle and the threshold for response. The larger the muscle the higher the threshold. This is reflected by the following facts (a) The grasshopper metathoracic tibial muscles are larger than any of the other muscles and have correspondingly higher thresholds. (b) Only in the metathoracic limb of the grasshopper is the flexor muscle smaller than the extensor and only in the metathorax of the grasshopper is the threshold lower for the flexor than for the extensor muscle. (c) The meso- and metathoracic muscles of the mantid are very small and correspondingly have low thresholds. The tibial muscles in the raptorial limbs of the mantid prothorax are larger and the thresholds for response in this case are as high as those for the grasshopper pro- and mesothoracic preparations. (d) The correlation between threshold and absolute muscle size was finally confirmed in the following manner. The metathoracic tibial extensor and flexor muscles of a very large female grasshopper and a very small male grasshopper were tested for thresholds, and then dried and weighed. The correlation between weight of muscle and original threshold recorded in Table 1.5 are very striking.

TABLE 1.5.

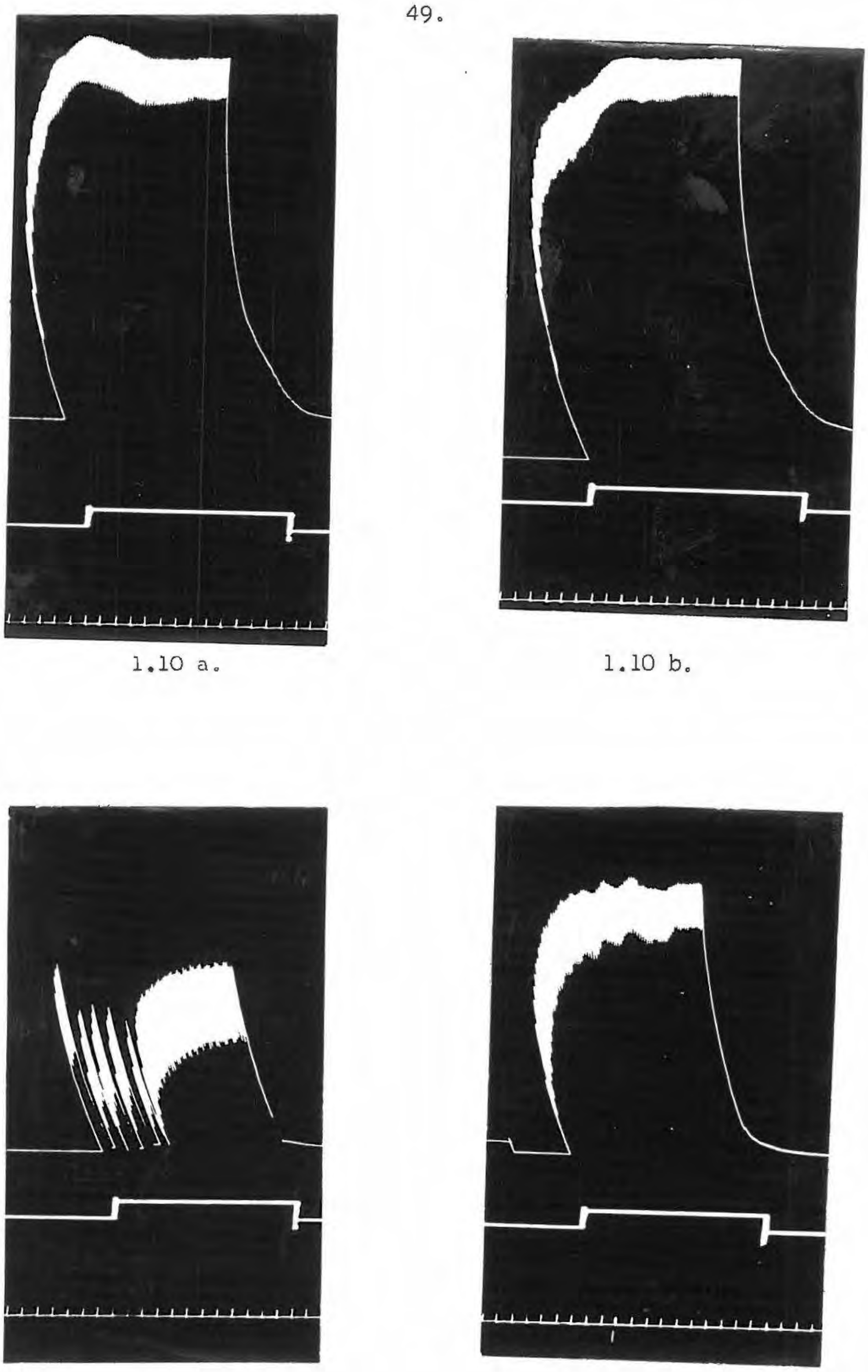
ANIMAL	SEX	MUSCLE	NERVE	MUSCLE DRY WT. (GRAMS)	THRESHOLD (VOLTS)
Maphyteus	Female	Extensor	5	0.0436	0.34
Maphyteus	Female	Flexor	5	0.0142	0.25
Maphyteus	Male	Extensor	5	0.0178	0.27
Maphyteus	Male	Flexor	5	0.0069	0.21

The results recorded in Table 1.5 suggest that the threshold for response is correlated with the number of final nerve endings on the muscle, the larger muscles possessing a larger number of end plates. This in turn may imply that the greater number of final nerve endings, the larger the axon leaving the ganglion. Certainly, as was shown in the anatomical study, the nerve fibre diameter does correlate with the size of the animal and thus presumably with muscle size. It seems clear that the axon diameter will be correlated with the number of muscle fibres innervated. It was, however, impossible to determine from the transverse section which of the numerous axons visible supplied a particular muscle; consequently this could not be pursued further.

2. THE EFFECTS OF CHANGES IN THE IONIC COMPOSITION OF THE SALINE ON THE MECHANICAL RESPONSES OF THE MUSCLES.

The innervation and mechanical responses of the tibial muscles in the grasshopper and the mantid having been established with some certainty, using a standard saline solution, the next step was to test the effects of different potassium concentrations on these responses. Three different procedures were adopted in these tests.

(i) Initially an extensive series of experiments was conducted, using grasshopper and mantid preparations, in which test salines were added to the opened thoraxes. The nerves innervating the tibial muscles were thus bathed in the salines. Test salines were made up according to Hoyle's (1953) formula. Two solutions A and B were prepared, A containing 140 mM potassium per litre and 10 mM sodium per litre, and B containing 0 mM potassium per litre and 150 mM sodium per litre. By mixing these two solutions in different proportions a range of saline solutions of different potassium ion concentration were prepared. Henceforth in the text, to avoid unnecessary repetition, a test solution containing, for example, 10 mM of potassium per litre will be referred to as a 10 mM potassium saline. At the start of each experiment mechanical recordings were made from the femoral muscles before the addition of any test saline. Subsequently the test solution was added and after 15 minutes the effect of the addition on the preparation was recorded. To guard against the possibility of the test solutions affecting the muscles in the femora directly by flowing into the leg, the limb was mounted in plasticine above the level of the thorax. The results of these experiments were, however, inconsistent and confusing. The results of one of the experiments are quoted here. With preparation (a) the responses of the muscle were recorded at a stimulation frequency of 10 per second before the addition of any saline (Trace 1.10a). 10 mM potassium saline was then added which resulted in a slight modification of the response of the muscle (Trace 1.10b). As could reasonably be



1.10 a.

1.10 b.

1.10 c.

1.10 d.

TRACES 1.10 a, b, c and d. Effects of salines of different potassium concentration (when added to the opened thorax) on the mechanical responses of the metathoracic extensor tibiae muscle of *Maphyteus* with stimulation of nerve 5 (Preparation a). 10b with 10 mM potassium saline, 10c - 56 mM, 10d - 70 mM. 10a control in normal haemolymph. Stimulation frequency - 6 per sec. 15 min. lapse between each trace. Time trace: 1 sec.

expected addition of 56 mM potassium saline caused a decrease in the contraction level (Trace 1.10c). Subsequent addition of 70 mM potassium saline, instead of reducing the contraction height still further, caused an increase in contraction height (Trace 1.10d). 112 mM potassium saline completely blocked neural transmission in approximately 15 minutes.

In other preparations addition of 70 mM potassium salines had no effect. 112 mM potassium saline again blocked neural transmission. Thus the inconsistencies shown in these experiments were too great to allow detailed analysis of the effects of additions of potassium.

These experiments revealed, however, that neural transmission in the grasshopper and mantid is blocked almost immediately by the addition of 142 mM potassium saline. 112 mM potassium saline causes almost immediate neural block in the mantid while taking somewhat longer to block transmission in the grasshopper. Furthermore 70 mM potassium saline could block neural transmission in the mantid within a few hours while having little effect on the grasshopper preparation. In both cases the block produced with high potassium salines is reversible if low potassium salines are again added and the preparation left for some time. These results suggest that the axons of the mantid are more "exposed" or susceptible to high concentrations of potassium in the saline than those of the grasshopper. However, it is not clear how much of this difference in susceptibility between the two animals is to be related to differences in absolute size, and so conclusions may be drawn only somewhat tentatively.

(ii) In a second series of tests, saline solutions of differing potassium concentrations were injected directly into the femora of the grasshopper and mantid. In the majority of the grasshoppers 56 mM potassium saline was sufficient to produce a complete and apparently irreversible block to muscular contraction; in some animals 42 mM potassium saline was sufficient to block the action of the muscles. Injection of 28 mM potassium saline had no noticeable effect on the grasshopper muscles. With the mantid, injection of 42 mM potassium saline was always sufficient to produce a complete block, and in some cases injection of 28 mM potassium saline produced a block. In control animals the same quantity of low potassium concentration saline was injected into the femora. With grasshoppers and the majority of the mantids this control injection had no noticeable effect. In some of the mantids, however, the addition of the low concentration potassium saline to the femur was sufficient to produce a muscular block. It seems likely that this block is caused by the mechanical effects of saline injection, and does not necessarily reflect a nervous block brought about by the potassium ions. This snag was the cause of the eventual abandonment of these experiments.

No rigid conclusions can be drawn about the site of action of the salines, on injection into the femora. The results suggest that there may be a difference between the nerves in the thorax and those located more peripherally in the leg - the former being apparently better "protected" than the latter. It is possible that the larger nerves in the thorax have more efficient ionic "pumps" than those located peripherally in the leg. This suggestion must be regarded as being very tentative, however, as the differences may well be attributed to the fact that in one case the femoral muscles themselves are exposed to the saline, while they are not in the other case.

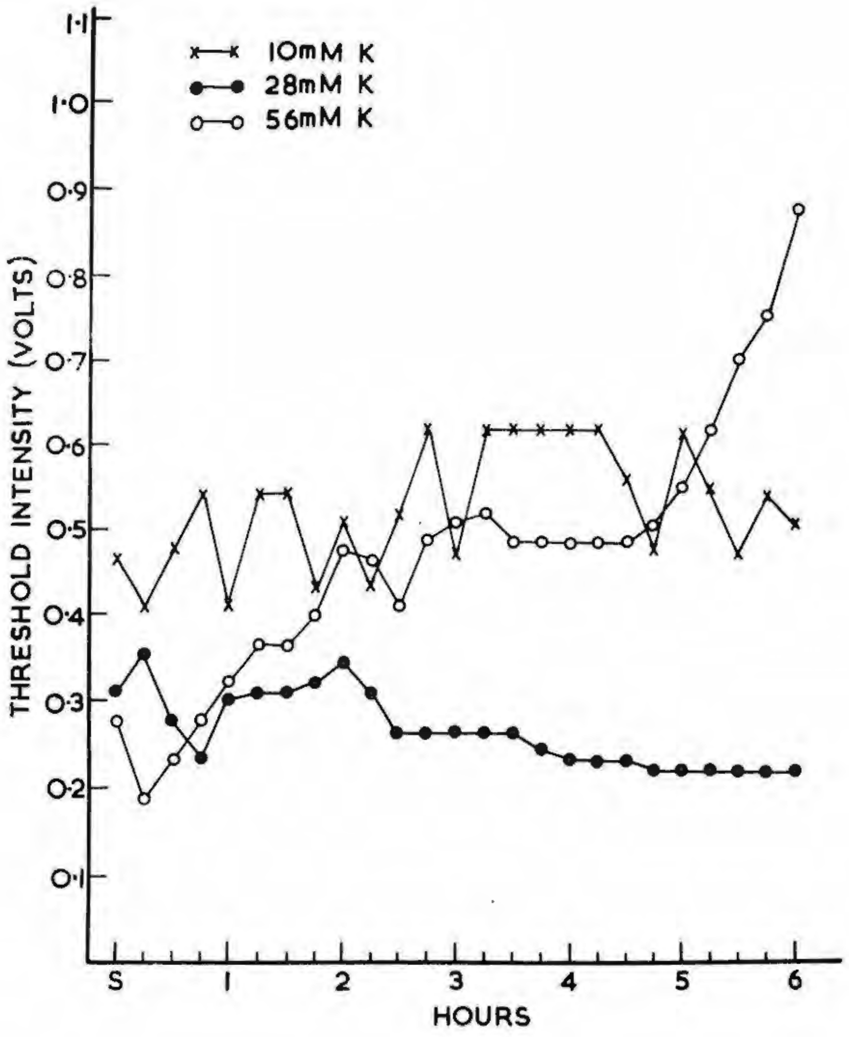
(iii) A third attempt to assess the effect of potassium rich salines on the neuromuscular preparations was conducted along the following lines. The thoracic nerves were exposed in the normal manner, after which enough cuticle was shaved from the femur to enable the flexor tibia muscle to be removed while leaving the extensor muscle and its nerve supply intact. A space remained in the femur above the extensor muscle surrounded by chitinous walls. The "trough" thus formed on top of the extensor muscle could be filled with test saline and the effects of this addition on the responses of the extensor muscle recorded. At the outset this seemed to have potential as a test preparation. Again, however, an accurate analysis was not possible as the results of these experiments were too inconsistent. In control experiments, using a grasshopper, the addition of standard Hoyle's saline to the femoral trough often had a marked effect on the mechanical responses. In other cases the addition of saline had no effect at all. An attempt to obtain uniformity of results was made by setting up preparations as follows. The flexor muscles from both metathoracic femora of one animal were removed. Each tibia was connected to levers, both of which wrote simultaneously, one above the other, on a smoked drum. Both nerves 5 were stimulated at the same instant with exactly the same stimulus. Frequently, before any test saline had been added, the limbs on both sides of the body reacted similarly to the stimulus. Occasionally, however, the two limbs react completely differently to an identical stimulus. As might be expected from the initial control experiments, addition of standard saline to the femoral troughs, sometimes but not always, affected the mechanical response of the two limbs. In one preparation addition of saline affected one leg and not the other. At times 56 mM potassium produced a reversible muscular block, though usually, addition of 70 mM potassium saline to the femoral troughs was necessary to produce a block.

Attempts to remove the tibial extensor muscle from the femur of the mantid failed. The femur was so weakened by the operation that it buckled when the extensor muscle contracted. Experiments of this type were consequently discontinued.

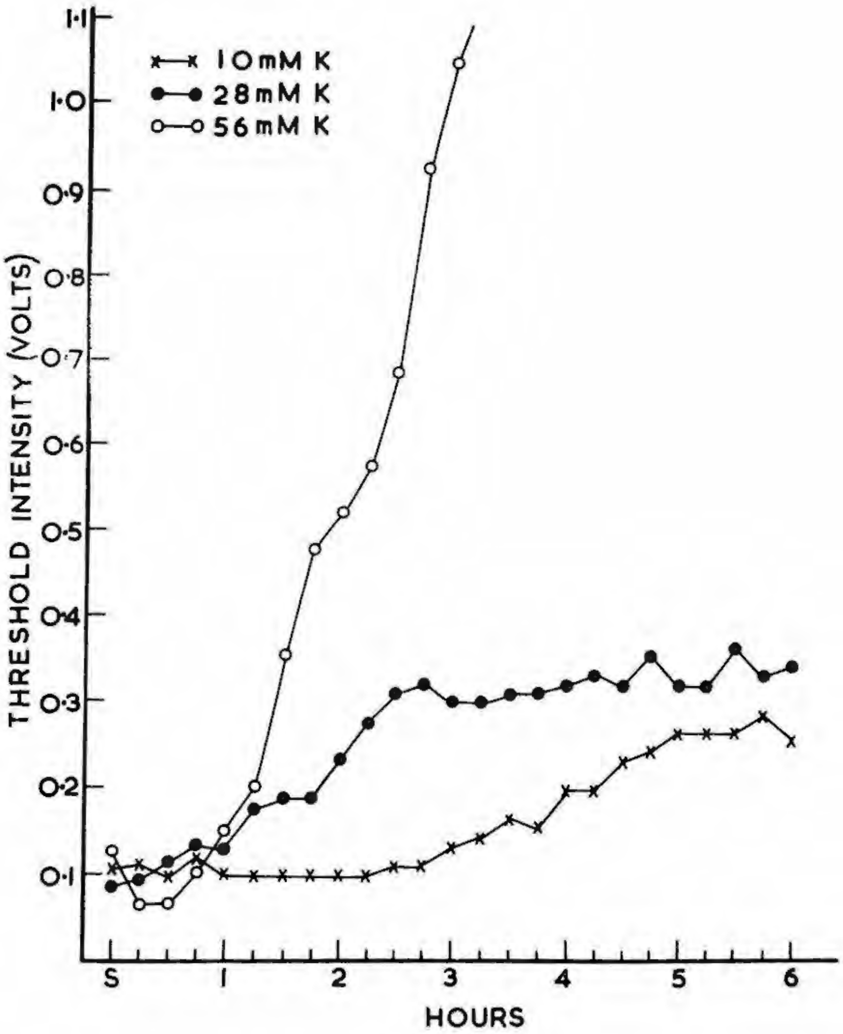
All these experiments emphasise the inadequacy of simple mechanical assessments of the effect of potassium rich salines on the muscle response. They do show, however, that there is a difference in the susceptibility to high potassium salines in the mantid and grasshopper. In all tests lower potassium concentrations were required to produce adverse effects on neural transmission and muscular contraction in the mantid than in the grasshopper, the implications of this will be discussed later. It is not clear how far these differences relate to the size of the animal, although it is obvious that for an accurate analysis of the effects of high potassium saline a quantitatively more precise method must be adopted. During the course of the experiments such a method suggested itself. Throughout the experiments it was noted that addition of different salines to the preparations, while not necessarily affecting the mechanical response, had a clear effect on the threshold stimulus intensity. These threshold changes could easily be measured by monitoring the stimulus, which was reaching the nerve, on the cathode ray oscillograph. Thus the threshold for response could be directly and accurately read off from the cathode ray oscillograph.

3. THE EFFECTS OF CHANGES IN THE IONIC COMPOSITION OF THE SALINE ON THE THRESHOLD FOR MUSCULAR RESPONSE.

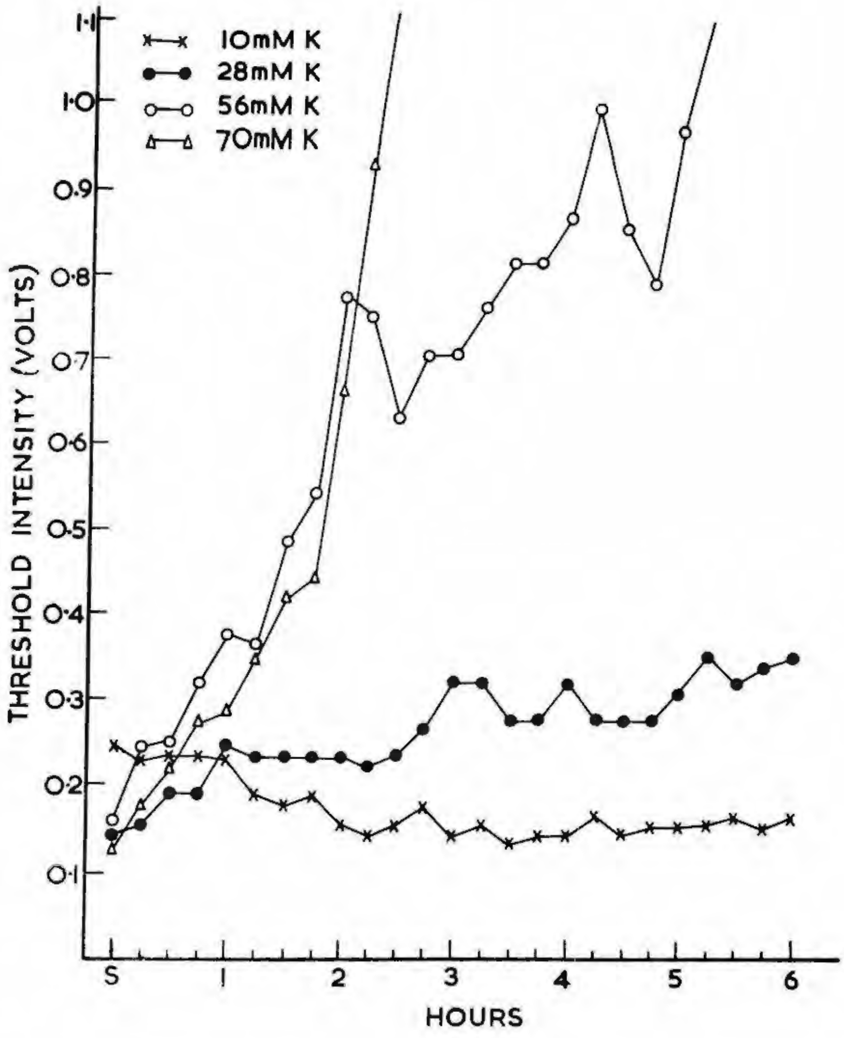
A standard procedure was followed throughout these experiments. The normal preparation was set up as rapidly as possible and the threshold stimulus intensity for muscle response was measured immediately (Points S in Graphs 1.1 to 1.8). The test saline solution was then introduced into the thorax. The precautions described previously were taken to ensure that none of



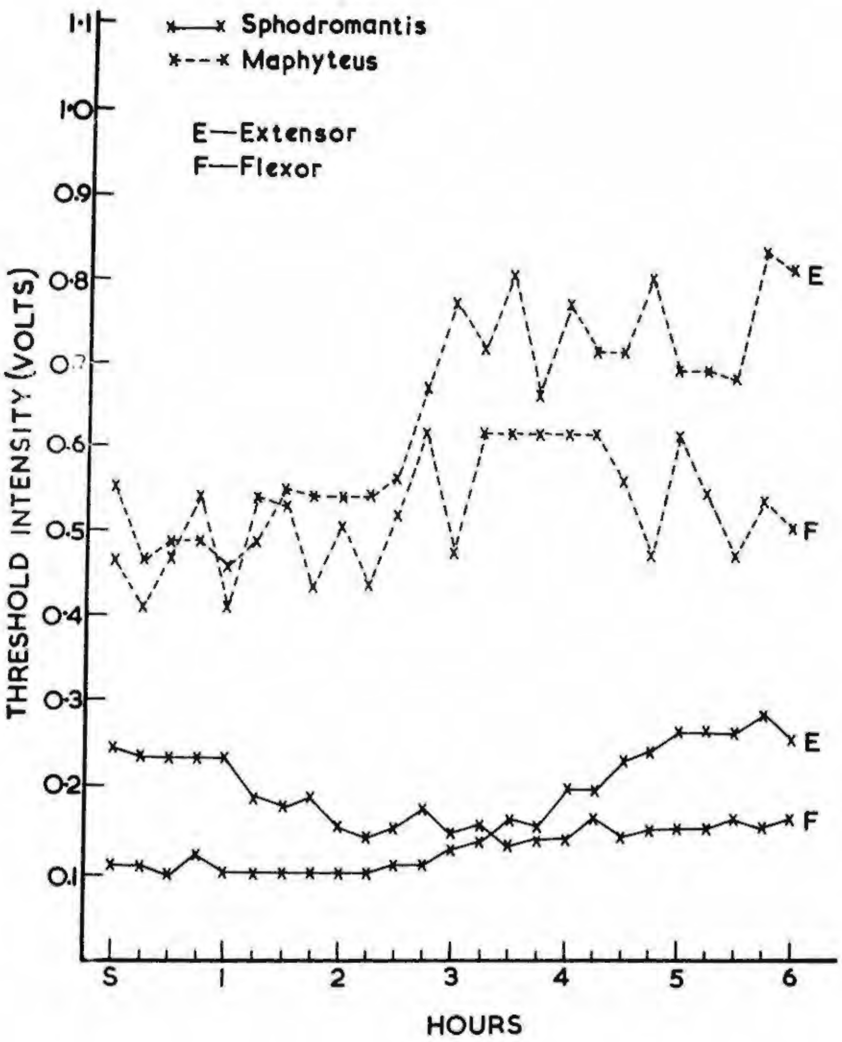
GRAPH 1.2. Effects of potassium in the saline on the threshold for response of the grasshopper flexor tibiae with stimulation of nerve 5. (Preparation c).



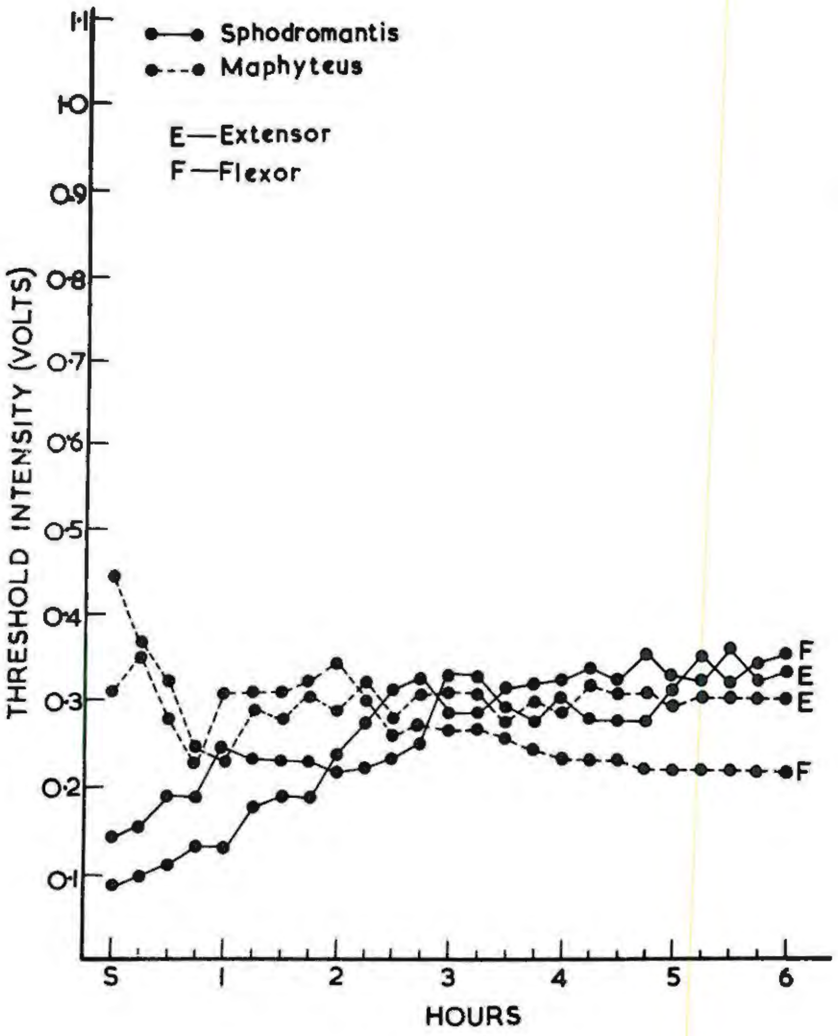
GRAPH 1.3. Effects of potassium in the saline on the threshold for response of the mantid extensor tibiae with stimulation of nerve 3. (Preparation f).



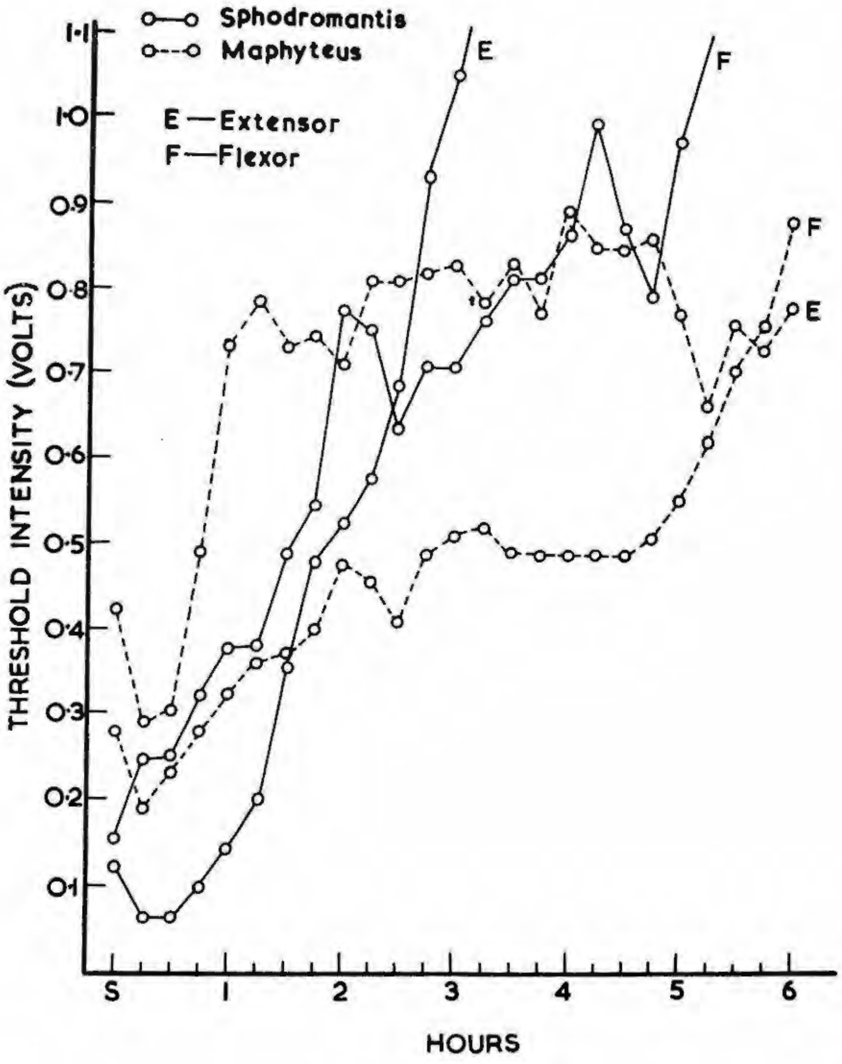
GRAPH 1.4. Effects of potassium in the saline on the threshold for response of the mantid flexor tibiae with stimulation of nerve 5. (Preparation g).



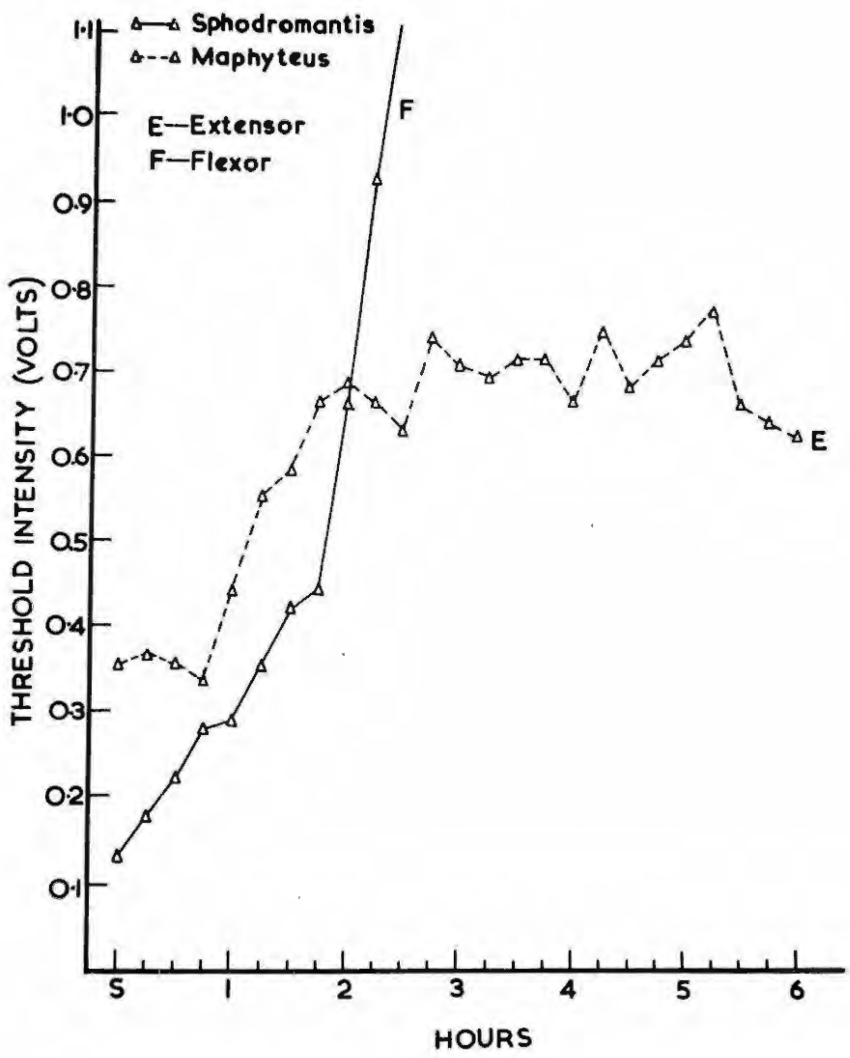
GRAPH 1.5. Thresholds for response, with nervous stimulation, in the extensor and flexor tibiae muscles of the grasshopper and mantid using a 10 mM potassium saline.



GRAPH 1.6. Thresholds for response, with nervous stimulation, in the extensor and flexor tibiae muscles of the grasshopper and mantid using a 28 mM potassium saline.



GRAPH 1.7. Thresholds for response, with nervous stimulation, in the extensor and flexor tibiae muscles of the grasshopper and mantid using a 56 mM potassium saline.



GRAPH 1.8. Thresholds for response, with nervous stimulation, in the extensor and flexor tibiae muscles of the grasshopper and mantid using a 70 mM potassium saline.

the saline flowed into the limbs themselves. Subsequently, every 15 minutes, the nerve to be stimulated was lifted out of the saline bath. A threshold reading was taken, and the nerve lowered back into the thorax. Particular care was taken to ensure that no tissue from the thoracic region was adhering to the nerve or to the electrodes. If such adhesion occurred the insulation thus produced markedly increased the threshold. Initial control experiments showed that with a suitable saline the threshold remained constant for at least 24 hours and often for as long as 36 hours.

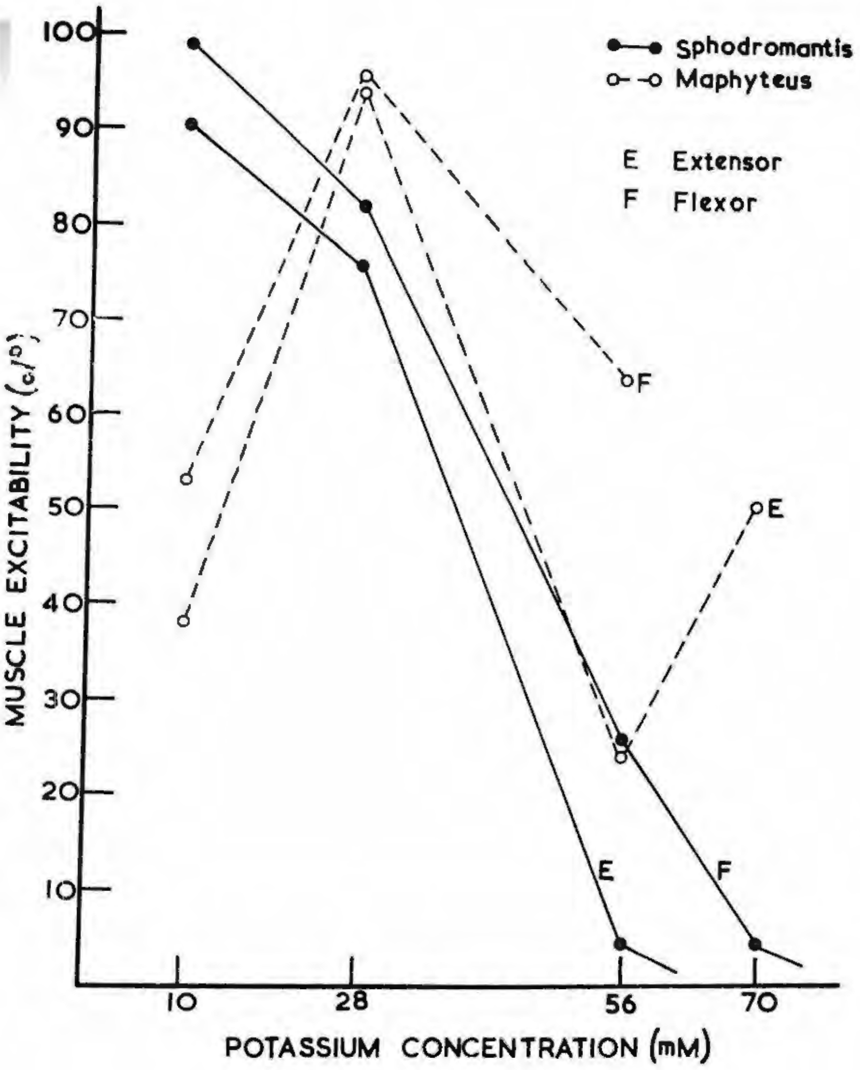
The results of these experiments are recorded in Graphs 1.1 to 1.8 in which the threshold stimulus intensity is plotted against time in hours. The effects of different potassium concentrations on the thresholds for response in the extensor and flexor tibiae muscles of Maphyteus and Sphodromantis are shown in Graphs 1.1 to 1.4. To facilitate comparison the same results are expressed in a different manner in Graphs 1.5 to 1.8, where the responses of one particular muscle with different potassium concentration salines are assembled in one graph.

(a) In the case of the mantid tibial extensor and flexor muscles, the stimulus intensity required to produce a response increases with an increase in potassium concentration. The lowest recorded thresholds for mantid muscles are with 10 mM potassium saline. 28 mM potassium saline raises the threshold, while with 56 mM potassium and 70 mM potassium salines the threshold increases very rapidly. After immersion in 56 and 70 mM potassium, for a few hours, nervous conduction is practically blocked. With the tibial extensor and flexor muscles of the grasshopper the situation is different. Here the lowest thresholds recorded are in combination with the 28 mM potassium saline. 10 mM, 56 mM and 70 mM potassium salines produce uniform threshold increases

(b) The responsiveness of the grasshopper muscle is not affected as adversely by high potassium concentration salines as that of the mantid. In the grasshopper 56 mM potassium and 70 mM potassium salines do not completely block nervous conduction as they do within a few hours in the mantid. The fact that the grasshopper and mantid muscle is still responsive, even when nerve conduction is blocked, can be shown by stimulating the muscle tissue directly.

The overall picture of the results contained in Graphs 1.1 to 1.8 is diagrammatically summarized in Graph 1.9 where the percentage muscle responsiveness (or excitability) is plotted against the potassium ion concentration in the saline expressed in millimoles. The percentage muscle excitability was calculated at a point four hours after the start of the experiments. (This point was taken purely arbitrarily). 100% excitability was taken as the lowest threshold ever recorded for the muscle in question. From Graph 1.9 then it is evident that the nerves supplying the tibial muscles in these two animals are adapted to operation in haemolymphs of differing potassium concentration. The grasshopper is adapted to operation in approximately 28 mM potassium saline and the mantid to operation in approximately 10 mM potassium saline, a situation which makes functional sense, as it is widely accepted that predaceous insects have a lower haemolymph potassium concentration than phytophagous forms.

In all, these experiments have shown the axons of the entomophagous Sphodromantis to be more susceptible to high external potassium ion concentrations than those of the phytophagous Maphyteus. What explanation can be offered to account for these differences? Suppose that in the nerves of both animals, beneath the level of the perilemma or surrounding the axons themselves, there is some sort of ionic "barrier" (of the type which Hoyle has postulated as being in the perilemma itself) which is responsible



GRAPH 1.9. Excitability (%) of the extensor and flexor tibiae muscles of the grasshopper and mantid with nervous stimulation and with differing concentrations of potassium in the surrounding saline. For further explanation see text.

for the exclusion of ions from the immediate environment of the axons. Suppose further that this barrier may act firstly, as a purely passive barrier to the entry of ions, and secondly that it has incorporated in it active metabolic "pumps" for the exclusion of ions. From this we may postulate two possible explanations to account for the difference in susceptibility of the axons to ions. It might be that the passive element of the barrier is equally well developed in Maphyteus and Sphodromantis, but that in Maphyteus the postulated ionic pumps are more effective or more numerous, and thus afford the axons greater protection, than those in the mantid. Alternatively it may be that the pumps are equally effective and of equal abundance in both animals but that the passive element of the barrier is more highly developed in phytophagous than in predatory forms. (This situation is represented diagrammatically in Plate 1.17).

If ionic pumps are responsible for the noted differences in the susceptibility of the axons in phytophagous and predatory forms it could be expected that addition of drugs (which will not be affected by the pumps) to the haemolymph would affect the axons of these two forms equally. It will, however, be shown in the following preliminary investigation into the action of drugs upon predatory and phytophagous forms, that the axons of phytophagous forms are less adversely affected by drugs than those of the mantid, and consequently it would seem that the postulated ionic pumps are equally effective in both forms but that the passive element in the barrier is more highly developed in the plant feeders than in the predators.

4. THE ACTION OF DRUGS ON PREDATORY AND PHYTOPHAGOUS FORMS.

The actions of two drugs, Gamma amino butyric acid,(GABA), and Picrotoxin, (PCTX), on predatory and phytophagous forms have been investigated.

The choice of GABA and PCTX as test drugs was prompted primarily by many recent reports on the effects of GABA on the neuromuscular system of Crustacea (Bazemore et al. 1957, Brockman and Burson 1957, Edwards and Kuffler 1957, Kuffler and Edwards 1958, McLennan 1957, Werman et al. 1960, and Kravitz et al. 1962), as well as the effects of GABA and PCTX on the nervous and muscular systems of the Crustacea (Elliot and Florey 1956, Robbins and van der Kloot 1958, van der Kloot and Robbins 1959, Grundfest et al. 1958, van der Kloot et al. 1958, Robbins 1959, Hichar 1960). In essence these workers find that GABA produces an inhibitory effect on the crayfish neuromuscular preparation and that this inhibition is blocked by PCTX.

The effects of these drugs on insects were investigated in three ways: (i) standard saline, containing the drugs, was injected into live animals and the action of the drugs noted. (ii) dilutions of drugs in saline were introduced into the thoraxes of the mantid and the grasshopper in similar preparations to those previously described; and (iii) saline and drugs were injected into the femora of mantids and grasshoppers and their action on the mechanical response of the muscles recorded.

(i) Saline solutions containing concentrations of 10^{-3} to 10^{-6} GABA or PCTX were injected in small doses (0.2 - 0.3 cc.) into healthy adult Maphyteus, Sphodromantis, Periplaneta and Ectrichodia. Control animals injected with larger doses of normal saline showed no ill effects. All four different animals react in a specific manner to both GABA and PCTX. The reaction to each drug is clearly defined and very different.

Injections of GABA at concentrations of 10^{-3} resulted in an almost immediate loss of tonus manifested by drooping antennae, and slackness of the limbs resulting in the abdominal and thoracic sterna being dragged along the substratum. This lack of tonus persisted and was followed by convulsive shivering and a very obvious decrease in mechanical efficiency. Eventually movement ceased and the animals would not respond to violent external stimuli (loud sounds, bright lights and vibrations) though they would respond very slightly to mechanical stimuli directly on the body. After 6 hours in this relaxed, almost moribund state, the animals show signs of recovery and, after 36 hours, in the majority of cases, revival was complete, the insects appearing to be normal. This loss of tonus produced by GABA points to a general inhibition of muscular activity, (or perhaps nervous activity) similar to that reported for the Crustacea.

Injections of PCTX at concentrations of 10^{-3} caused very rapid tetanus in all the muscles of the body and limbs. The abdomen "concertinad" together and the legs flexed strongly under the body. The tibial flexor muscles had the dominant effect in the limbs of all the insects, except for the metathoracic limb of the grasshopper. Here, the extensor muscle being stronger than the flexor, powerfully extended the hind limbs. In a short time convulsions set in and during this time the animal was hyper-sensitive to stimuli reacting violently even to the most gentle stimulation. Eventually the convulsions became more intense and death occurred from 6 to 15 hours after injection.

The effects of more dilute drug solutions on the animals were also tested. In all cases injection of 10^{-4} and 10^{-5} drug solutions produced the same symptoms as the 10^{-3} concentrations. With 10^{-4} and 10^{-5} concentrations of GABA the symptoms of poisoning took longer to develop and recovery was more rapid. In the case of 10^{-5} GABA injection full recovery took about six hours. It was noticeable, however, that injection of GABA in low concentrations had a more marked effect on the mantid and reduviid than on the cockroach or locust.

With 10^{-4} and 10^{-5} PCTX there was also a delay in the manifestation of symptoms after injection. Injection of 10^{-4} PCTX solutions invariably resulted in the death of the experimental animal. Death was delayed for about 36 hours in the case of the mantid and for 48 hours or more with the reduviid, grasshopper and cockroach. 10^{-5} PCTX injection killed all the mantids used, but this dilution of PCTX did not cause 100% mortality in the other three insects. On the whole fewer locusts and cockroaches died from the injections than reduviids. It was usually 48 - 80 hours before the animals died. These results are again indicative of a difference in susceptibility of phytophagous and predatory forms.

Injections of 10^{-6} solutions of GABA or PCTX had no noticeable effects on the mechanical efficiency or responses of any of the animals tested.

In several experiments 10^{-3}, 10^{-4} and 10^{-5} PCTX solutions were injected following an injection of GABA and after the symptoms of GABA intoxication had appeared. This always resulted in the disappearance of the symptoms of GABA intoxication and the appearance of symptoms of PCTX poisoning which then took their normal course. Injection of 10^{-6} PCTX after the symptoms of GABA intoxication had appeared had no effect on these symptoms. Injection of GABA after the symptoms of PCTX poisoning had manifest themselves was not attempted.

(ii) Preparations were set up as previously described and the nerves in the thorax supplying the femoral muscles of the mantid and the grasshopper were bathed in salines containing different concentrations of GABA and PCTX. Solutions of 10^{-4} and 10^{-5} PCTX and GABA had surprisingly little effect on the nerves.

10^{-3} GABA also had little effect on the nerves, but 10^{-3} PCTX produced a rise in nerve threshold followed by an eventual transmission block. Although these experiments were conducted in a very superficial manner it was clear that 10^{-3} PCTX produced a more rapid rise in nerve threshold and subsequently a quicker transmission block in the nerves of the mantid than in those of the grasshopper.

(iii) Direct injection of 10^{-3} GABA saline into the femora resulted in a loss of tonus in the tibial extensor muscles, although the effect was not very marked. Injection of PCTX, even in low concentrations (10^{-4} and 10^{-5}) produced very rapid tetanus in the muscles and in some cases injection of 10^{-6} PCTX into the femur of the mantid resulted in a tetanic contraction from the muscles.

These very preliminary experiments suggest that the axons of predatory insects are less well protected from drugs in the haemolymph than those of the plant feeders. Following from the discussion on page 66 (Plate 1.17) it would seem that the postulated ionic pumps in both phytophagous and predatory forms are equally effective but that the passive element in the barrier is probably more highly developed in the plant feeders than in the predators.

SUMMARY.

1. It has been suggested by Hoyle (1953) that the nerve sheath surrounding the peripheral nerves of insects serves as a protective barrier to exclude potassium ions in the haemolymph from the immediate environment of the axons.

2. This has led to the postulate that in entomophagous insects this barrier might be far less developed than that in phytophagous insects since it is known that the blood potassium concentration is far lower in predatory insects than that of plant feeding insects.

3. The gross anatomy and histology of the peripheral nerves innervating the tibial extensor and flexor muscles of a phytophagous insect, _Maphyteus leprosus_, an omnivore _Periplaneta americana_, and two predators, _Sphodromantis gastrica_ and _Ectrichodia crux_, are described.

4. It is shown that there is remarkable consistency in the thickness of the nerve sheath around nerves of comparable size in all four animals and that the larger nerves have the thicker nerve sheaths.

5. This leads to the conclusion that the differences in thickness of the nerve sheath in different insects correlates with mechanical factors and has no relation to protection of the nerve axons from the ions in the haemolymph.

6. This conclusion is in agreement with that of Treherne who has shown the nerve sheath to be freely permeable to ions in _Periplaneta_. Treherne's studies have also suggested the presence of a deeper barrier protecting the axons from ions in the haemolymph.

7. The study into the properties of this deeper barrier has been approached indirectly and a preliminary investigation of its properties has been made by comparing its efficiency in protecting

the axons against varying external potassium ion concentrations in the different insects being studied.

8. This investigation has shown the axons of the entomophagous Sphodromantis to be more susceptible to high external potassium ion concentrations than those of the phytophagous Maphyteus.

9. This leads to the conclusion that some barrier beneath the level of the perilemma is more efficient at excluding ions from the axons in phytophagous than in predatory forms.

10. It is postulated that this barrier may be acting either as a purely passive barrier to the entry of ions, or that it has incorporated in it active metabolic "pumps" for the exclusion of ions. Both possibilities may be true.

11. Preliminary experiments on the action of drugs on predatory and phytophagous forms suggest that the passive element in the barrier is more highly developed in the plant feeders and that there may be no significant difference in the action of the metabolic pumps in the two forms.

PART 2.

STUDIES ON THE HISTOLOGY, AND CERTAIN ASPECTS OF THE NEUROMUSCULAR PHYSIOLOGY OF THE FLIGHT MUSCLES IN *NUDAURELIA CYTHEREA CAPENSIS* STOLL.

RESUMÉ.

During the course of the work which has been described in Part 1, an outbreak of large saturniid moths (*Nudaurelia cytherea capensis* Stoll.) occurred in the Grahamstown area. It was felt that an investigation into the properties of the flight motor of this moth, which has an extremely low wing beat frequency, might be rewarding as our knowledge of the flight motor in insects is limited to those with very much higher wing beat frequencies than that of this moth. The anatomy, innervation and histology of the flight muscles of *Nudaurelia* are described and it is shown that the flight motor of this moth is functionally different ~~to~~ from that of other insects which have been investigated.

Further, *Nudaurelia* shows a characteristic warm-up fluttering of the wings prior to flight - this phenomenon has also been examined in the following investigation. This study has yielded information about the location of a warm-up centre in the central nervous system of this moth.

PART 2.

INTRODUCTION.

The neuromuscular mechanisms of insect flight have attracted the attention of physiological workers for many years. However, owing mainly to the practical difficulties involved in devising suitable preparations with such small animals our knowledge of the subject is still rudimentary. The insects which have received the most attention in this respect are those with a very high wing beat frequency and asynchronous flight (that is an asynchronous relationship between the frequency of muscle action potentials and wing beats, a situation which is functionally associated with fibrillar muscles, McCann and Boettiger 1961), for example the Diptera, (Boettiger and Furspan 1950 and 1952, Boettiger 1957, Pringle 1949, 1954 and 1957, Sotavalta 1953 and many others); the problem here has been to determine how wing beat frequencies of up to 1200 cycles per second are achieved with an apparently conventional neuromuscular apparatus. Insects with a lower wing beat frequency and synchronous flight (that is a synchronous relationship between the frequency of muscle action potential and wing beats, associated with non-fibrillar muscle types, McCann and Boettiger 1961) have been comparatively neglected (Buchthal, Weis-Fogh and Rosenfalck 1957, Ewer and Ripley 1953, Neville 1960, Weis-Fogh 1956, Riegert 1962). In this investigation the neuromuscular physiology of the flight motor of a lepidopteran with a very low wing beat frequency was studied.

Furthermore very little attention has been paid to the central basis of flight in insects (Dingle 1961 and Wilson 1961) and there appears to have been no study made of the central nervous basis of the preliminary "warm-up" which often precedes flight (Krogh and Zeuthen 1941). The moths used in the present investigation show a very pronounced "warm-up" and consequently offer ideal material for an investigation of this phenomenon.

MATERIAL AND METHODS.

For the greater part the insect used in this investigation was the very large saturniid moth *Nudaurelia cytherea capensis*, Stoll. the adult of which occurs locally in pine plantations for approximately two months of the year (May and June). These emperor moths are very heavy and have large broad wings: wing spans of 12-13 cm. have been recorded for the larger females. In flight the wings are flapped slowly and laboriously in contrast to the fairly high wing beat frequency of the sphingid moth *Theretra capensis* Linn, which has been studied for comparative purposes. *T. capensis*, like other hawk moths, has narrow wings and is a very agile, swift flier. Both moths were collected when available and used as soon as possible after capture - neither lives well in captivity, surviving for a maximum of three to four days in the laboratory.

The techniques employed were as follows:-

(i) Recording of muscle action potentials from the dorsal longitudinal muscles during flight was a relatively simple matter. The dorsal portion of the thorax was first de-scaled using a coarse paint brush. This operation is most unpleasant but most essential as it enables the electrodes to be placed in the same region of the thorax in all subsequent experiments. The moth's legs were then strapped, with lumps of plasticene, very firmly to a plate in the middle of a sliding micromanipulator. There appears to be no overwhelming tarsal reflex in this animal as it flies adequately, although not continuously, with its tarsi strapped to the substratum. The wings were trimmed to ensure that the wing beat was unhampered by the surrounding apparatus. Decreasing the load by amputating the wings in this manner makes no significant difference to the wing beat frequency. Recordings were made with platinum electrodes inserted into the thorax to a depth of about 3 mm., usually just to one side of the mid-dorsal line, and connected through a preamplifier to one channel of a double beam oscillograph. The oscilloscope camera was operated at a speed of 6" of film per second. In addition a standard gramophone crystal pick-up head was lowered onto the thorax of the tethered insect and led directly into the other channel of the oscilloscope. This recorded the distortion of the thorax during flight. Care was taken to ensure that the pick-up needle only came into contact with the thorax when the latter was maximally buckled dorsally, during the downstroke, otherwise the needle pierced the cuticle of the thorax.

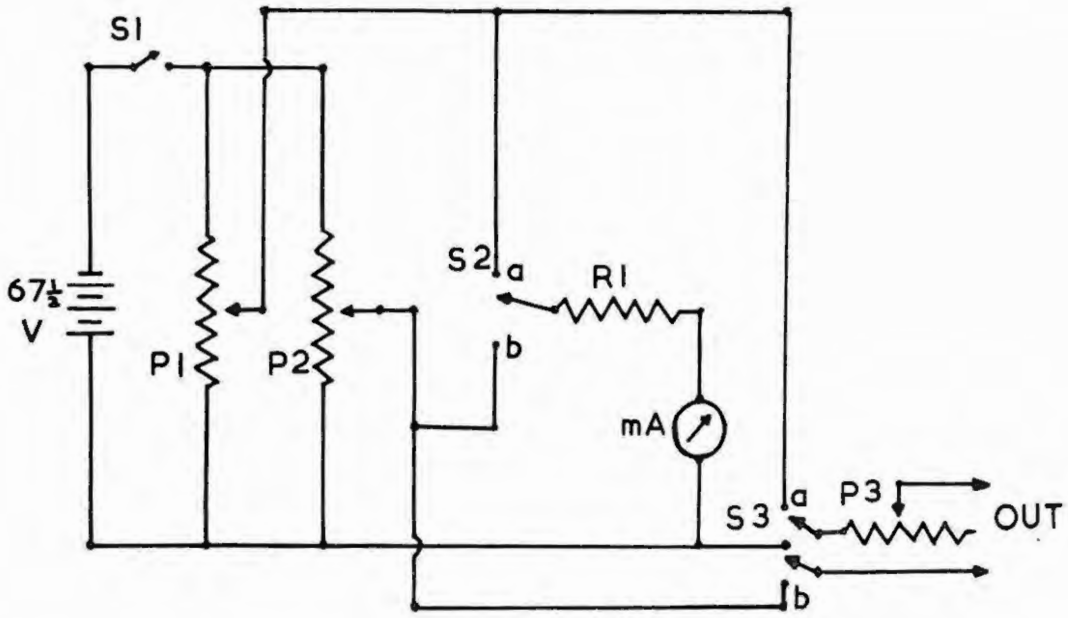
Flight is easily initiated by pinching the antennae or abdomen with forceps or blowing hard on the animal. Usually under such provocation the moth will flap its wings violently for a few strokes and then start to "warm-up" and eventually normal flight ensues. If the laboratory is kept fairly cool the animal will "warm-up" for longer periods before commencing normal flight. Normally, however, the laboratory was kept at a temperature of 23-25°C and at a relative humidity between 70 and 80%. The life of the preparation was prolonged at this humidity.

(ii) When it was necessary to stimulate the nerves or muscles in the thorax or observe the reactions of the dorsal longitudinal muscles directly through a binocular microscope the following technique was adopted. The thorax was de-scaled as before and the animal decapitated and dissected under the binocular microscope. The wings, dorsal longitudinal muscles and dorso-ventral muscles were removed from the one side of the thorax while leaving the nerves, muscles and wings of the other side intact. The animal was then mounted on a platform on the micromanipulator in such a way that the intact wing was free to move and could easily be observed. This was achieved by cutting a suitably sized hole in the platform through which the wing could pass and flap freely beneath the platform. With this arrangement it was possible to see exactly what wing movements occurred when certain muscles or nerves were stimulated and at the same time observe the precise movements of the very large dorsal longitudinal muscles. Before stimulating any particular nerve it was ligatured with hair and severed from the ganglion.

For A.C. stimulation Eads' stimulator described in Part 1 was used. The investigation into the central basis of "warm-up" necessitated the building of a D.C. stimulator, the circuit of which is illustrated in Plate 2.1. This circuit proved satisfactory in operation and has the advantage that the polarity of stimulation can be altered without altering the intensity of the stimulation delivered.

Finally it is necessary to record that no artificial saline was used for any of these preparations as it was found that there was sufficient haemolymph in the hemisected animal to keep the muscles moist for at least a few hours. It was found that the use of Hoyle's (1953) saline affected the longevity and responses of the muscles adversely.

(iii) The histological methods employed in the study of the flight muscles were fairly straightforward. Four fixatives were tried for muscle fixation, namely Carnoy, Bouin, Alcoholic Bouin and Flemming-without-acetic. Of these only Alcoholic Bouin and F.W.A. were at all satisfactory, the latter giving, by all appearances, more faithful fixation. The muscles were fixed either in the thorax and dissected out subsequently, or else, dissected out of the living animal and dropped into fixative. The first method was far superior to the latter. The histological schedules followed for preparation of the muscles were as follows:-



DIRECT CURRENT STIMULATOR

PLATE 2.1. The Direct Current Stimulator.

Components:- 67½ Volt Eveready Super 3 Battery. One ON-OFF switch S1. One double pole, single throw switch S2. One double pole, double throw switch S3. Two balanced 25 k potentiometers P1 and P2. One 25 k potentiometer P3 for fine output adjustment. One 19 k fixed resistance R1. One milliammeter mA.

With Alcoholic Bouin : Fix for ½ to 1 hr.

Rinse several times in 70% Alcohol.

90% Alcohol for ½ hr. (Several changes).

Absolute alcohol for ½ hr. (Two changes).

50% Cedar wood oil/50% Absolute alcohol for ½ hr.

Cedar wood oil - until cleared - usually about $^1/_4$ hr.

Wax 55° for $^3/_4$ hr. in vacuum embedder (three changes).

With Flemming-without-acetic :

Fix for 20 hr. in fresh fixative.

Running tap water for 3 hr.

30% Alcohol for 2 hr.

50% Alcohol for 2 hr.

70% Alcohol for 2 hr.

90% Alcohol for 6 hr.

Absolute alcohol for 2 hr.

50% Cedar wood oil/50% absolute alcohol for about $^1/_4$ hr.

Cedar wood oil - until cleared - usually about $^1/_4$ hr.

55° Wax for $^3/_4$ hr. in vacuum embedder (three changes).

All the sections were stained with Heidenhain's Iron Haematoxylin.

RESULTS.

It is convenient to divide the discussion of the results into three parts (i) anatomy and histology, (ii) neuromuscular physiology, and (iii) the central nervous basis of "warm-up".

(i) Anatomy and Histology.

Dissections of Nudaurelia under the binocular microscope have revealed much of the gross anatomy of the wing muscles - the dorsal longitudinal muscles in particular - and their innervation. Plate 2.2 is a camera lucida drawing of the hemisected thorax of Nudaurelia viewed from a dorso-lateral angle and Plate 2.3 a photograph of a dissection to show the arrangement of the muscles and the ganglia. It is to be noted that the dorsal longitudinal muscles (DLM) are divided into five large bundles numbered ventrodorsally 1-5, and that two sets of ventral longitudinal muscles (VLM) must be removed in order to expose the ganglia and associated nerves. Plate 2.4 is a drawing of the ganglia and the main nerves associated with them drawn from a dorsal aspect. Attention is called to the nervous connection N1 between the prothoracic ganglion and nerve N2, the significance of which will become apparent later; also to the fact that the dorsal longitudinal muscles are innervated by nerve NDL while the tergo-sternal muscles are innervated by three main nerves, only one of which, NDV, is shown in the diagram. Nuesch in a series of papers (1952, 1953, 1954, 1955 and 1957) has made a very extensive study of the morphology of the thorax of the saturniid moth Telea polyphemus Cr. and it would appear that the thoracic anatomy of both Nudaurelia and Theretra are practically identical to that of Telea.

Apart from the gross anatomy of the thorax there are two other aspects that should be investigated. Firstly it is desirable to know something of the axonal distribution to the flight muscles. This problem has been approached indirectly by Nuesch (1952) in Telea by cutting nerve trunks in the pupa and noting which muscles fail to develop in the adult; these results he has subsequently corrected and they are reproduced by Pringle (1957). Neuromuscular studies on Nudaurelia indicate that some of Nuesch's results are correct but suggest that others are erroneous. Lack of fresh material after this point was appreciated has prevented the necessary histological study

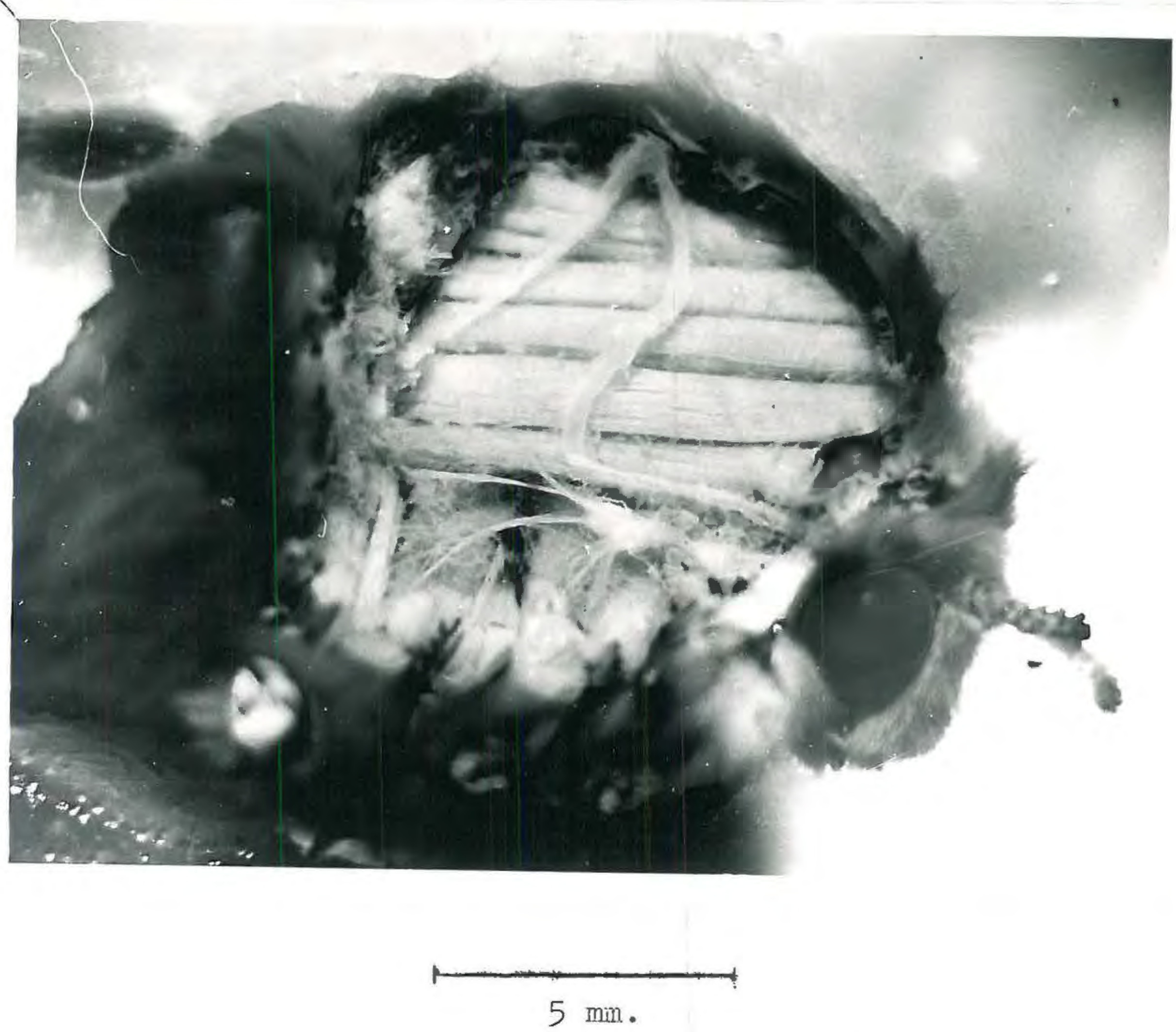


PLATE 2.3. Photograph of a dissection of Nudaurelia from a dorso-lateral aspect showing the position of the five dorsal longitudinal muscle blocks drawn in Plate 2.2, the heart, and the pro- and pterothoracic ganglia. Note that the two sets of ventral longitudinal muscles (VLM) illustrated in Plate 2.2 have been removed to reveal the ganglia.

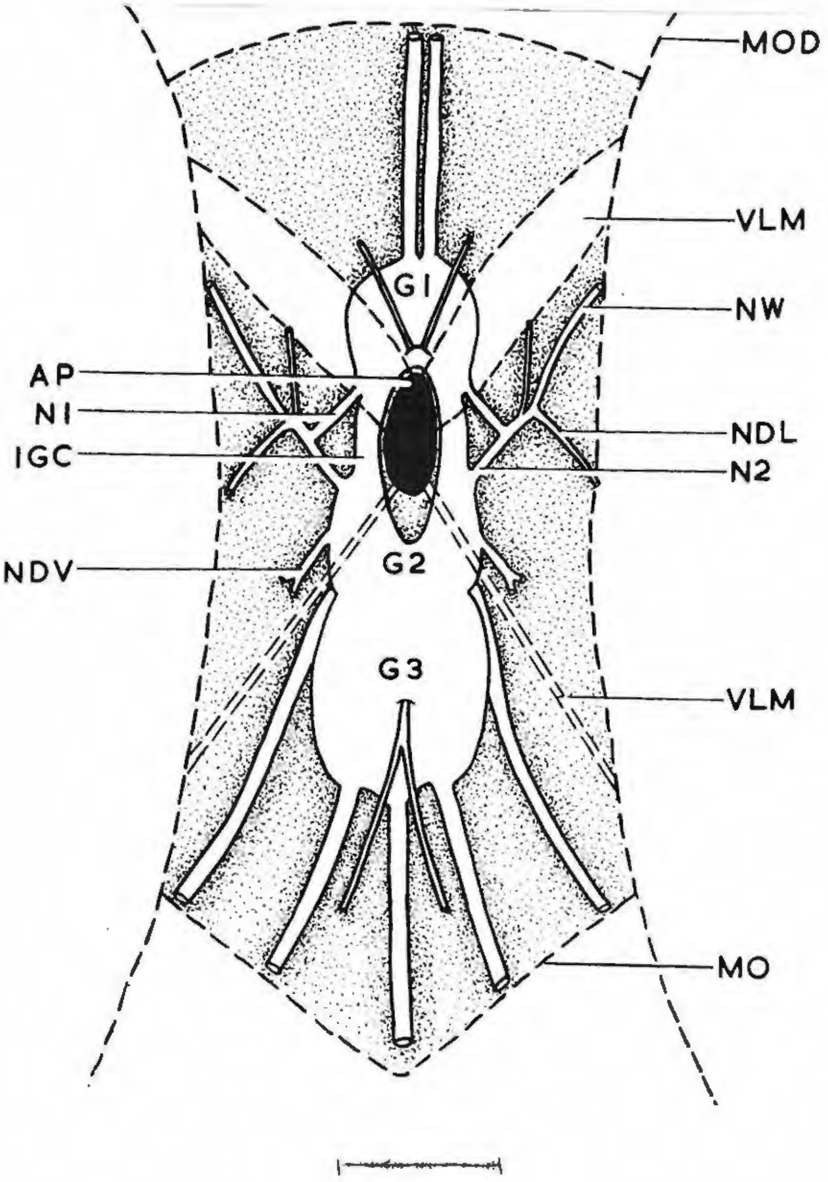


PLATE 2.4. Drawing of the thoracic ganglia of Nudaurelia from a dorsal aspect showing the main nerves, and the position of the ventral longitudinal muscles (VLM) which obscure the ganglia in Plate 2.2. G1, G2 and G3 are the pro-, meso- and metathoracic ganglia respectively. AP is a large apodeme (spina) upon which the ventral longitudinal muscles VLM originate. MOD and MO represent the broad outline of other muscles that had to be moved to one side and trimmed to reveal the ganglia. NDV is one of the nerves innervating the tergo-sternal flight muscles. There are two other nerves innervating these muscles but they are not drawn as they run almost ventrally from G2 and cannot be seen in this view. NDL is the only nerve that innervates the dorsal longitudinal flight muscles and NW innervates the wing. Nerves N1 and N2 are mentioned in the text. IGC is an interganglionic connective.

from being undertaken. A discussion on Nuesch's results is given later.

Secondly Tiegs (1955) and others have recognised several types of histologically distinct wing muscle in insects and it was necessary to determine which type of wing muscle *Nudaurelia* possesses.

In order to determine the histological category to which the muscles of *Nudaurelia* may be referred, it is primarily essential to decide the status of the "cells" so apparent in the transverse sections of the emperor moth muscle fibre (Plates 2.5 to 2.10.).

Consider first Tiegs's (1955) nomenclature in his discussion of the histological appearance of insect flight muscles. A muscle can be divided into units called muscle fibres, each of which is ~~are~~ ensheathed by a thin sarcolemma; the status of these muscle fibres is quite clear. The muscle fibres are usually gathered together in loose groups - a condition shown by *Nudaurelia* (Plate 2.11). The terminology applying to the muscle fibres themselves is often confusing and deserves some discussion. According to Tiegs each muscle fibre is subdivided into smaller units called fibrils and these run as individual entities throughout the length of the muscle fibre (diagrammatically shown in Plate 2.12). The fibrils are bound together in the fibre by the sarcoplasm which contains granular bodies called sarcosomes. Running in a network through the sarcoplasm are strands of a "dark interstitial sarcoplasm". In transverse section, of a muscle fibre, this network of "dark insterstitial" material can be seen to divide the fibres up into small polygonal areas, each of which encloses a number of fibrils. These small areas (which vary in size from about 2 - 8 microns) are defined as Cohnheim areas. The interstitial substance in the sarcoplasm that delimits the

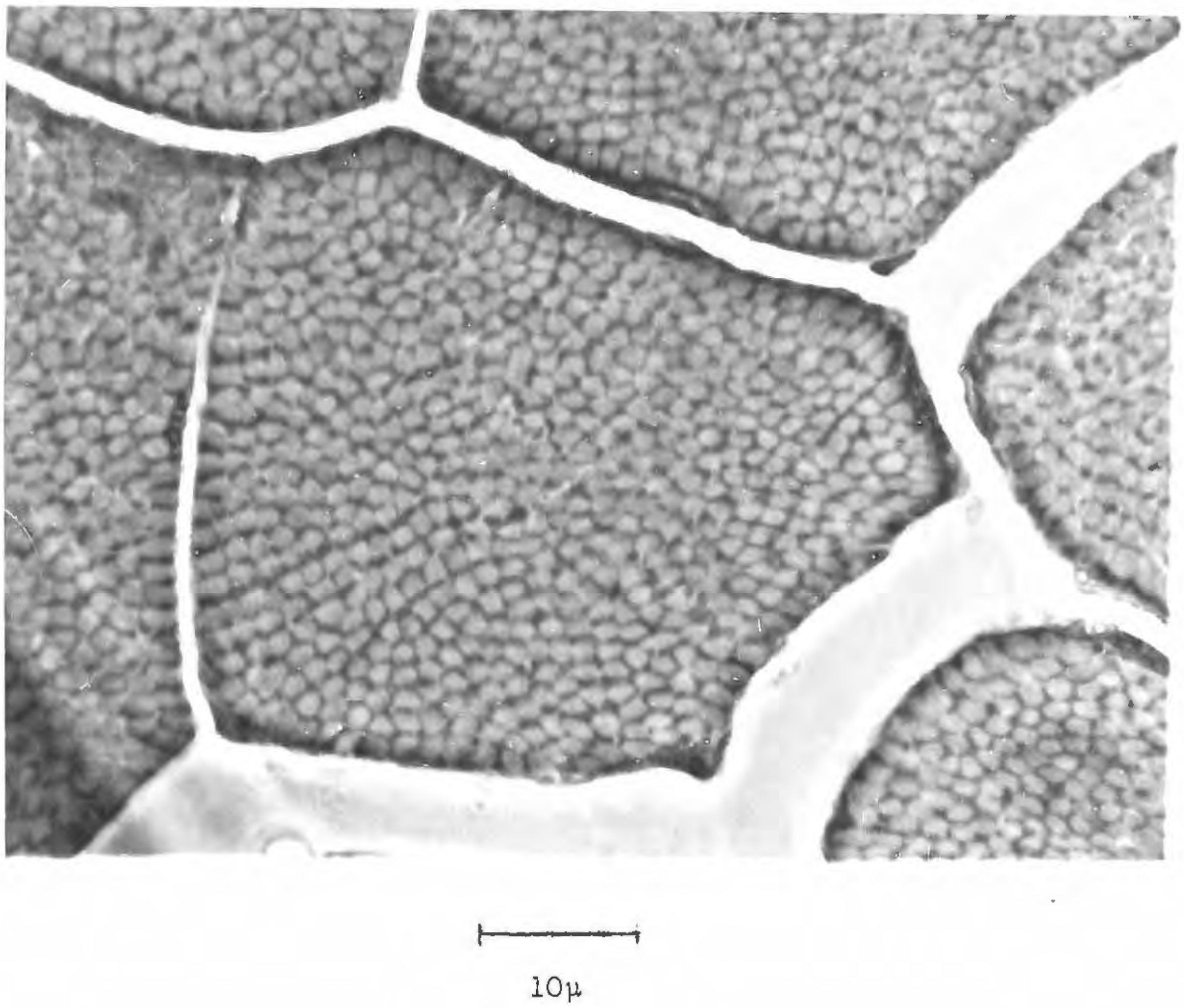


PLATE 2.5. Photomicrograph of muscle fibre of Nudaurelia under phase contrast. The muscles were fixed in FWA, sectioned at 4 microns and stained in Heidenhain's Iron Haematoxylin. Note the honey-comb arrangement of the "cells" (fibrils see text) within the fibres. Note also the presence of nuclei around the periphery of the fibre just beneath the sarcolemma, and the striking difference between these sections and those shown in Plates 2.7, 2.8, 2.9 and 2.10.

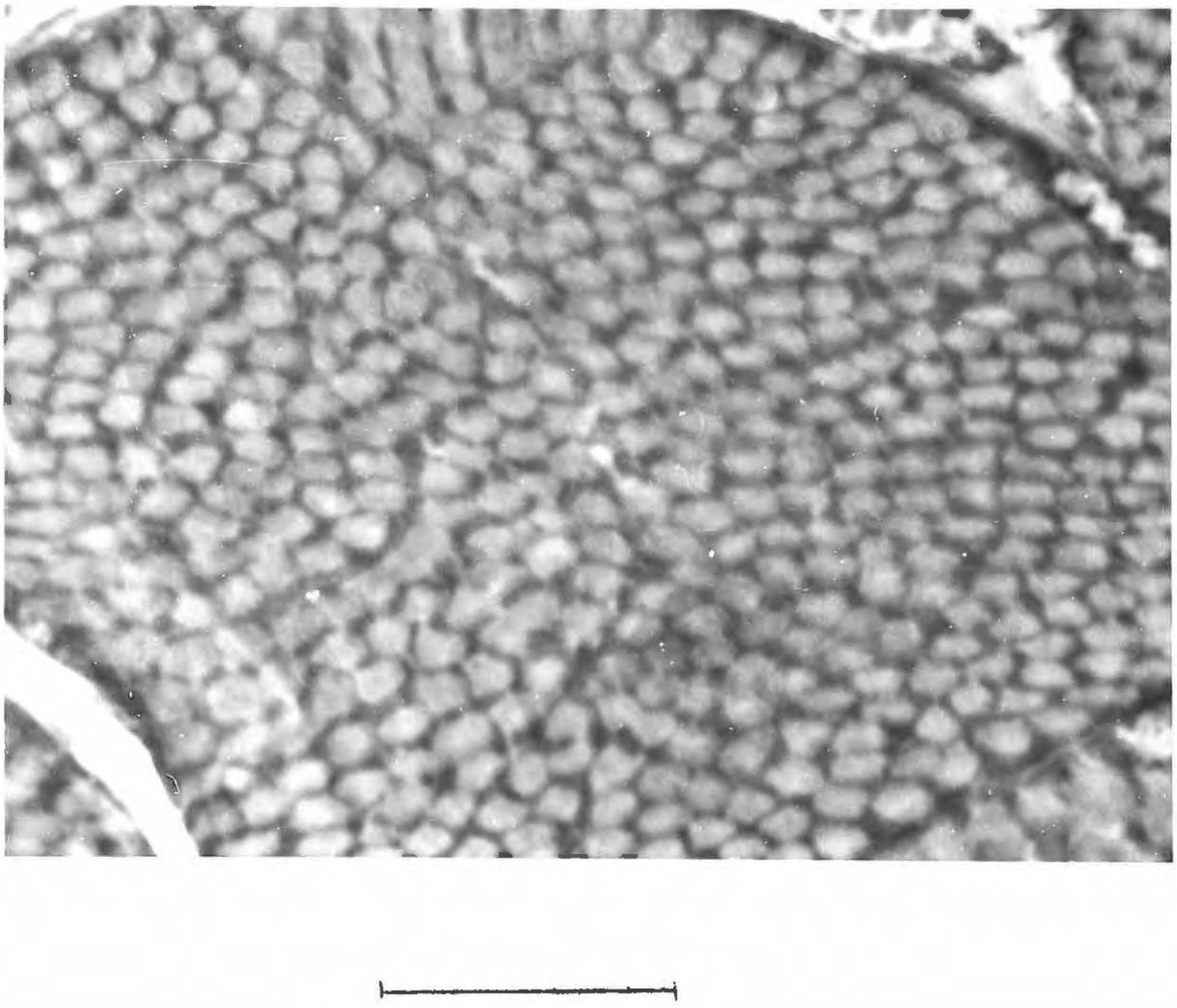


PLATE 2.6. Legend as for Plate 2.5. Note that this photomicrograph is at a greater magnification than Plate 2.5 and is for comparison with Plate 2.9 (see text).

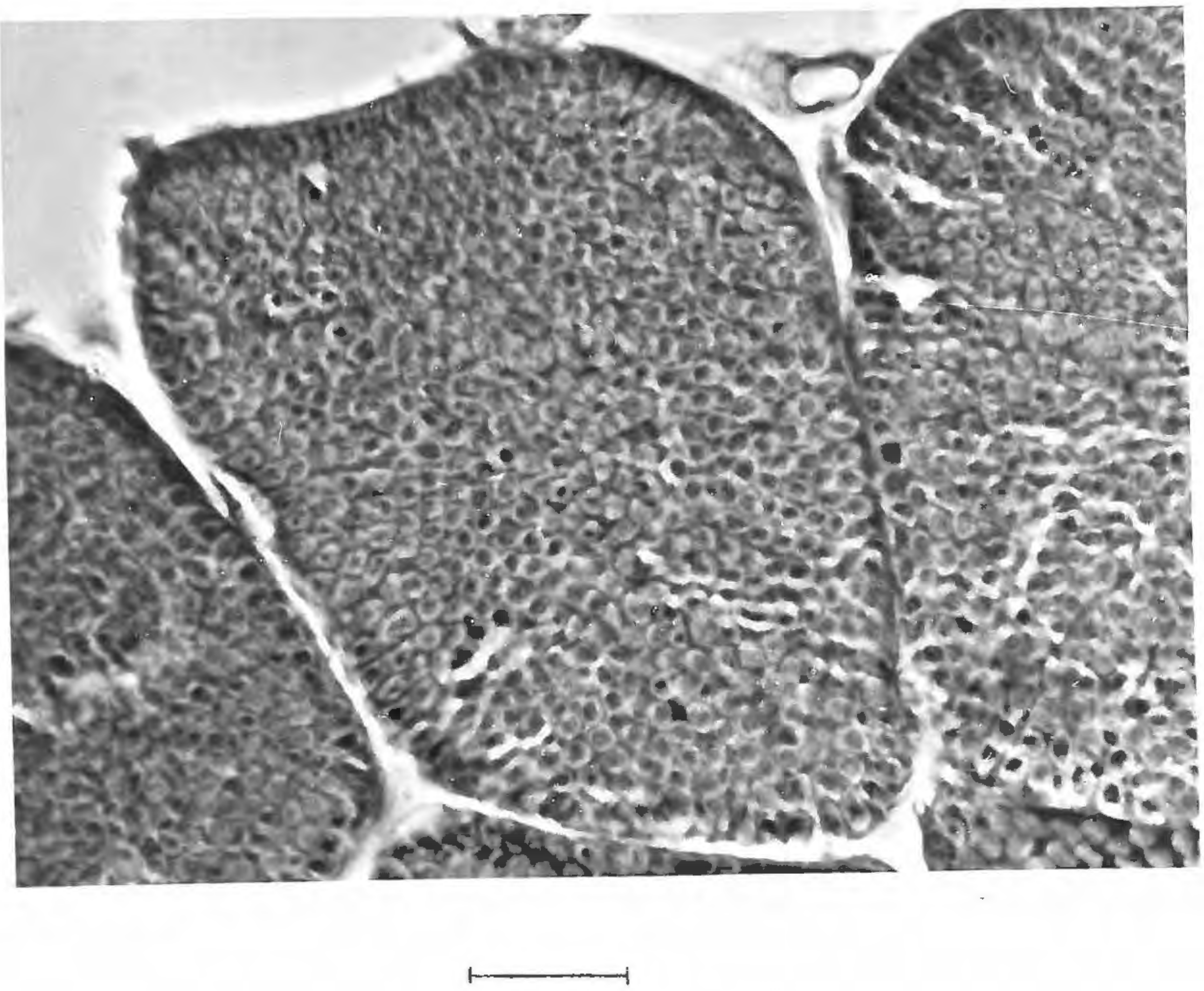


PLATE 2.7. Plates 2.7, 2.8, 2.9 and 2.10 are photomicrographs under phase contrast of cross-sections (4 microns thick) of the dorsal longitudinal muscle fibres of Nudaurelia. The photomicrographs illustrate the difference in appearance depending apparently on the state of fixation. All the muscles in these photomicrographs were fixed in Alcoholic Bouin and should be compared with the photomicrographs of the same muscles fixed in FWA shown in Plate 2.5. All the sections were stained in Heidenhain's Iron Haematoxylin. See text for an explanation on the significance of these plates.

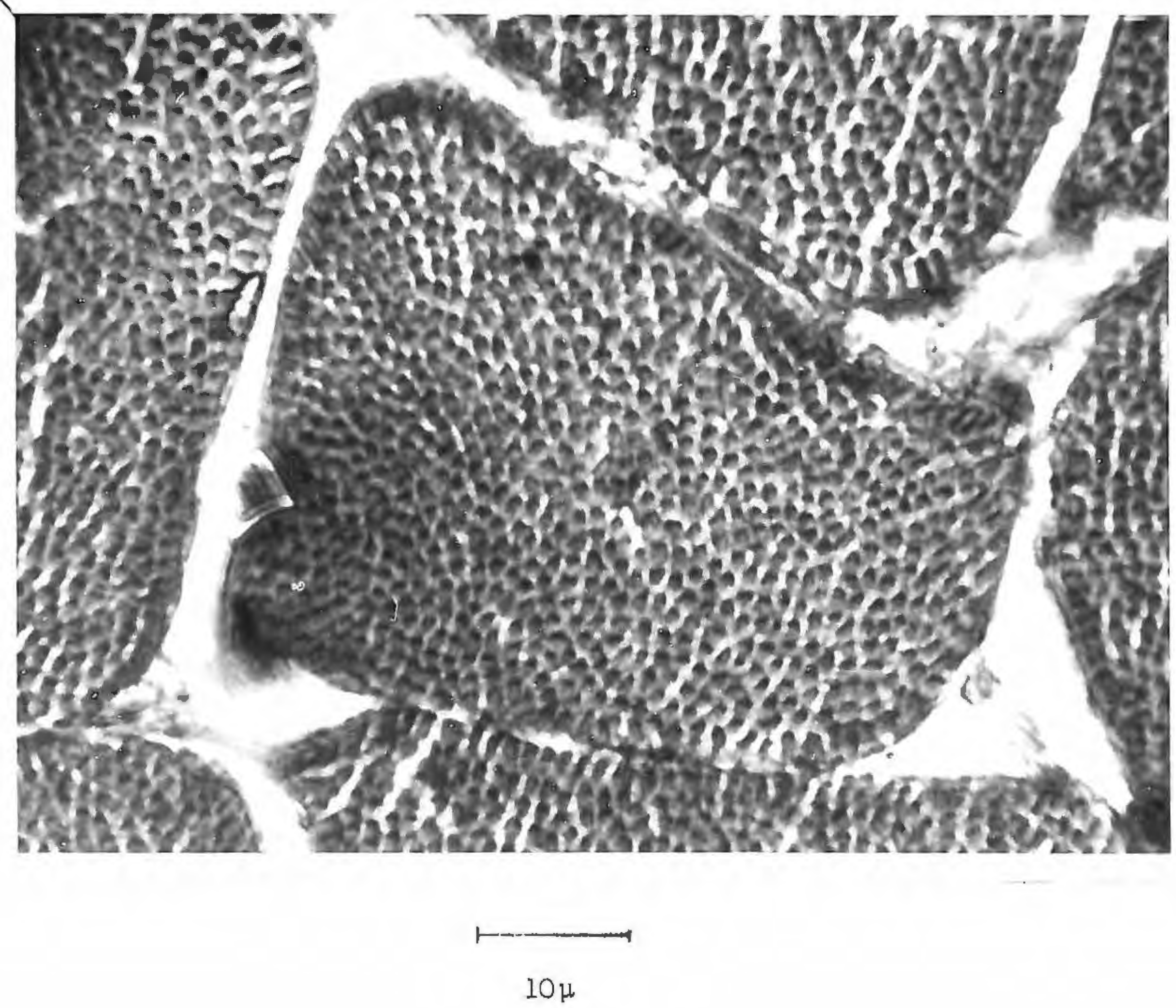


PLATE 2.8. Legend as for Plate 2.7.

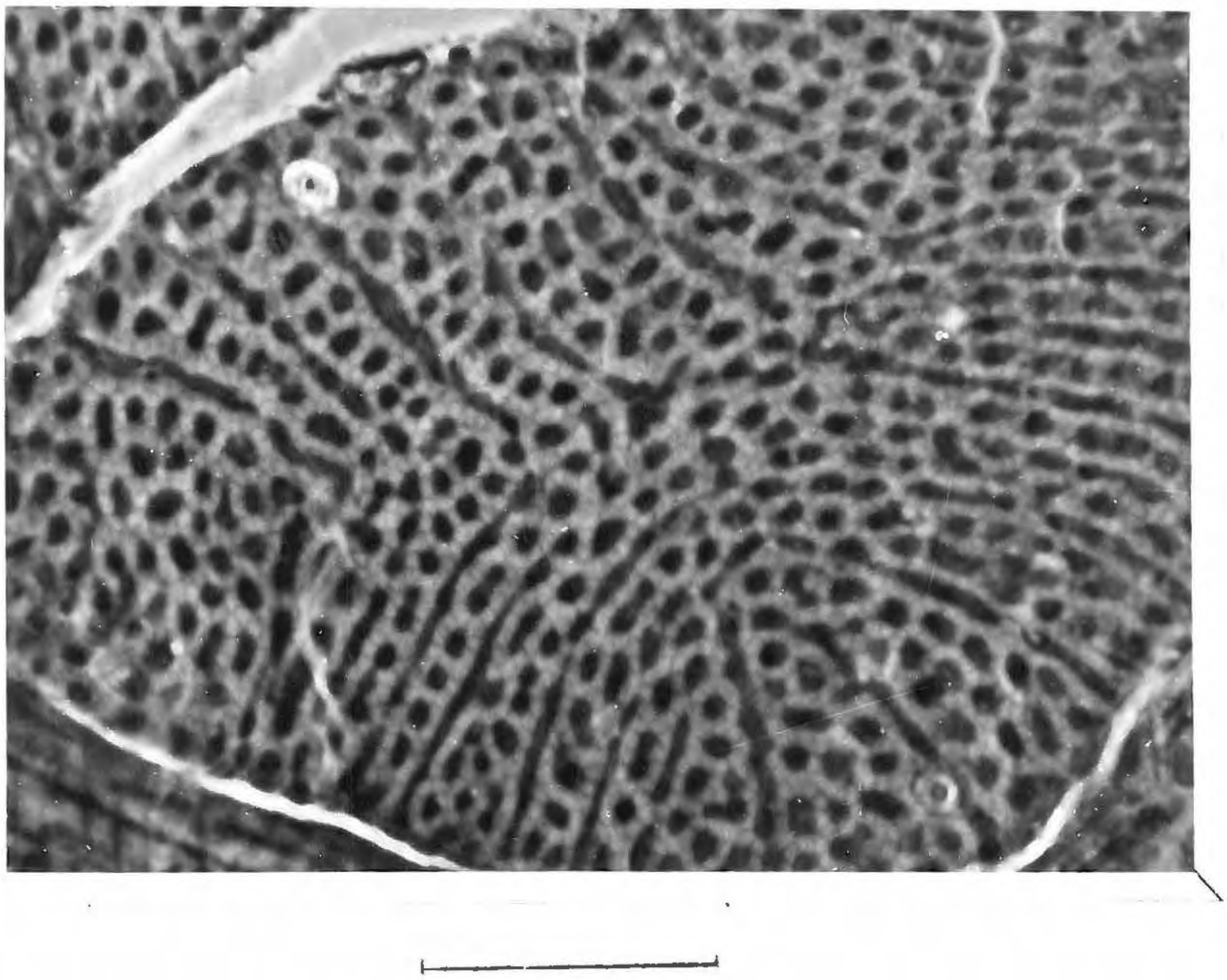


PLATE 2.9. Legend as for Plate 2.7. Note that Plate 2.9 is at a larger magnification than Plates 2.7, 2.8 or 2.10.

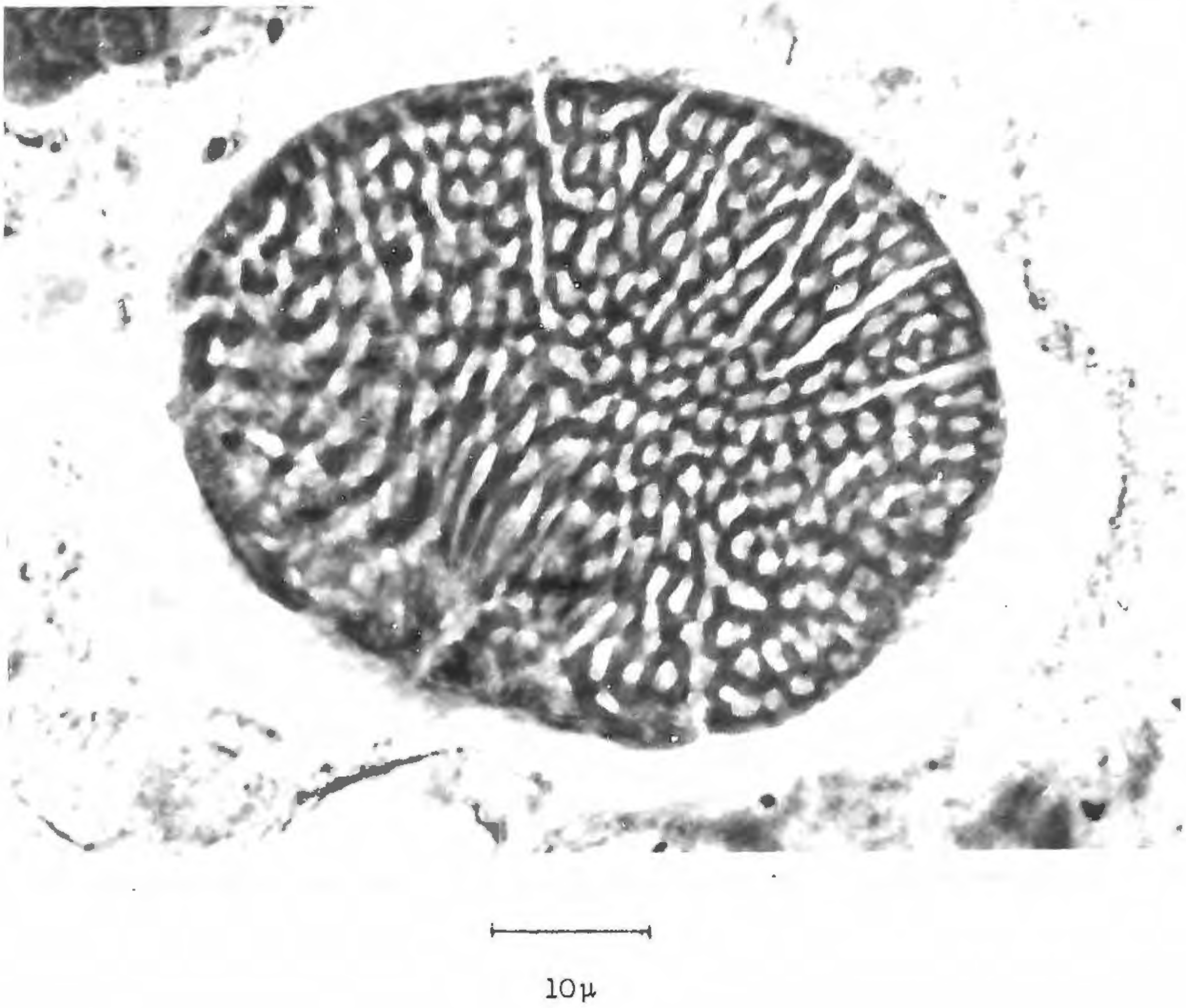


PLATE 2.10. Legend as for Plate 2.7.

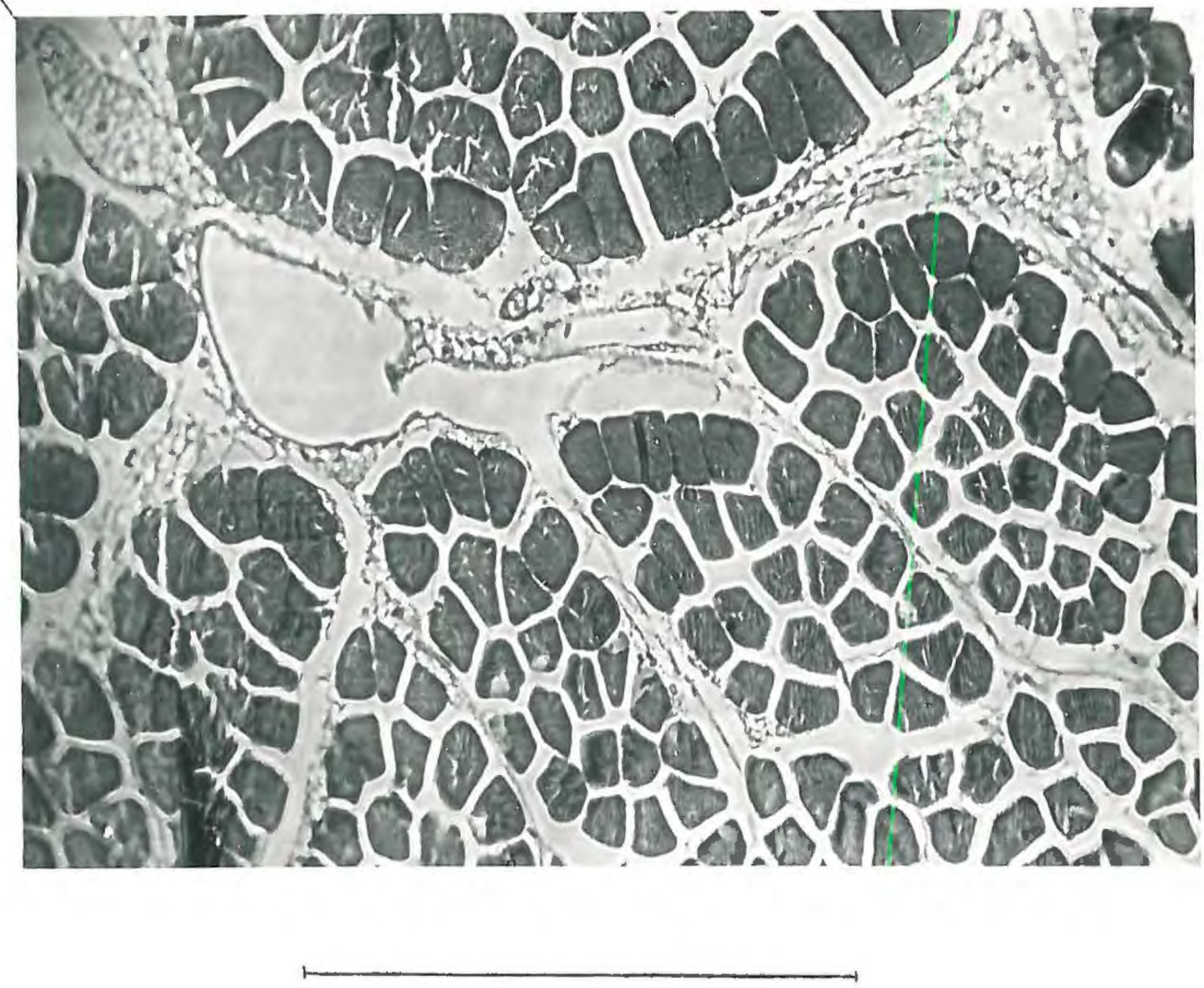


PLATE 2.11. Low power photomicrograph of a portion of *Nudaurelia* dorsal longitudinal flight muscle in transverse section. The photomicrograph, taken under phase contrast, shows the loose cohesion of the muscle fibres into groups.

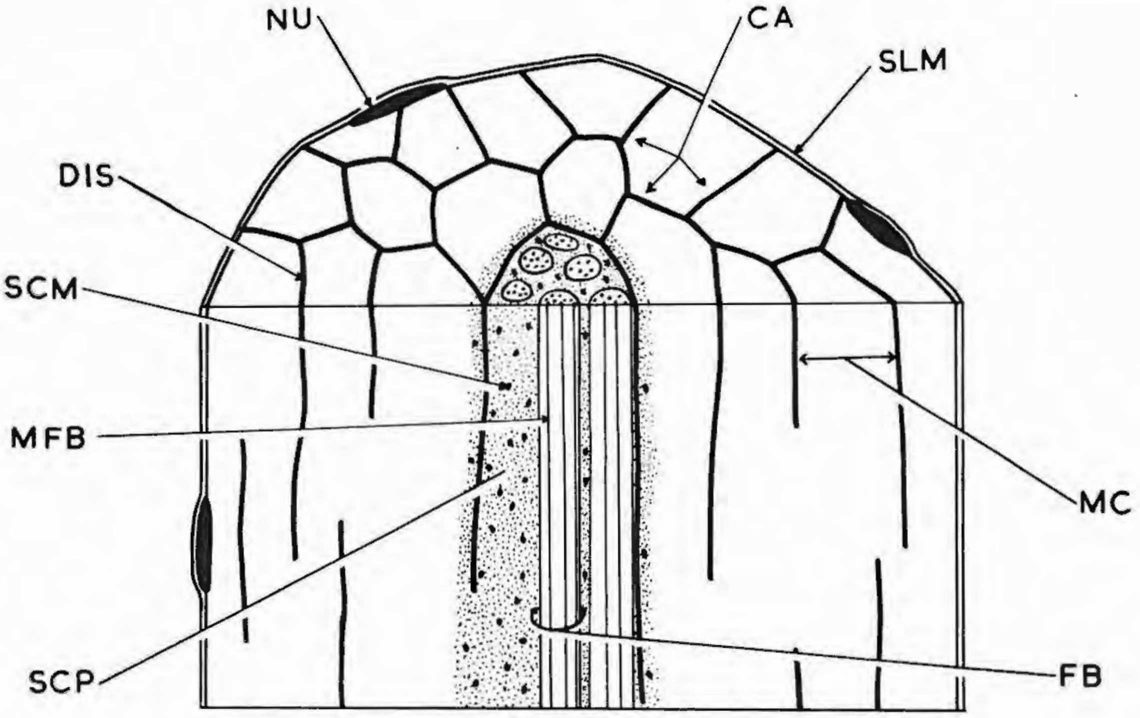


PLATE 2.12. Diagrammatic representation, in longitudinal and transverse views, of a muscle fibre following Tiegs's nomenclature. CA - Cohnheim area; DIS - Dark Interstitial Sarcoplasm; FB - Fibril or Sarcostyle; MC - Muscle Column; MFB - Myofibril; NU - Nucleus; SCM - Sarcosome; SCP - Sarcoplasm; SLM - Sarcolemma. The myofibrils are themselves held together in a "ground substance" within the fibrils.

the Cohnheim areas continues down the length of the fibre. In longitudinal section then, the areas enclosed by the interstitial substance are termed muscle columns - in other words the muscle columns are seen as Cohnheim areas in transverse section. The terms are actually used synonymously in some books (Bremer and Weatherford 1944). It is important to realise that because the dark interstitial substance is in fact a network, the muscle columns, unlike the fibrils, do not retain their individuality through the entire length of the muscle fibre. Furthermore, the interstitial substance surrounding the successive muscle columns do not run in a longitudinal series, but are irregularly arranged relative to each other. Consequently a single fibril, in its course along the fibre, may form part of two or more muscle columns.

What of the fibrils themselves? It is at this level that Tiegs's nomenclature differs from many writers in the field. Tiegs presents rather doubtful histological evidence to show that each of the fibrils (which vary in diameter from 0.5 - 5.0 microns in insects) are further subdivided into very fine "filaments" which he calls myofibrils. These myofibrils are bound together within the fibril by a "ground substance" and Tiegs envisages the myofibrils as the ultimate contractile units of the muscle. Tiegs further proposes that the term sarcostyle be given the new meaning of a fibril which is composed of subsidiary myofibrils. These concepts are diagrammatically represented in Plate 2.12.

This synthesis by Tiegs has done little to clear up the confusion in the literature, as authors have not taken readily to his scheme; in some cases muscle columns are confused with fibrils or the terms used synonymously. The vast majority of modern writers are clear on the latter distinction but use the term fibril and myofibril as synonyms, and where Tiegs's myofibrils are mentioned they are called myofilaments. (Edwards et al. 1954 a and b, Hodge 1955, Jordan 1955, Maximow and Bloom 1957, Ham 1957, Imms 1957, Pringle 1957, Smith 1960, 1961 a and b.) Tiegs's nomenclature will, however, be followed in the rest of this discussion.

There are at least five histologically distinct types of wing muscle found in insects.

(a) Tubular muscle - characterised by a central sarcoplasmic core, containing nuclei, in the fibre. This condition is typical of limb muscles but is found in the wing muscles of the Blattidae among the Orthoptera sensu lato.

(b) Secondly the muscles of the Odonata which are characterised by fibrils radiating from a central sarcoplasmic cone which contains the nuclei.

(c) The close packed or microfibrillar type (Boettiger 1960) is characterised by closely packed fibrils of less than 1.5 microns, in unfixed material, shrinking to 0.8 microns in fixed material. The nuclei are arranged around the periphery of the muscle fibre just beneath the sarcolemma. The Cohnheim reticulum is not retained except in some of the primitive Orthoptera (sensu lato - (Tettigonidae)). This type of muscle has been reported in the Orthoptera and in one Lepidoptera, a sphingid, by Keilich (1918).

(d) Fourthly, fibrillar muscle, found in Hymenoptera, Coleoptera and Diptera, which differs from the microfibrillar type in two respects:- (i) The fibrils are coarser in the fibrillar types, their diameter being greater than 1.0 microns and as much as 5.0 microns (Pringle 1957), averaging 3.0 microns in unfixed material and 1.5 microns fixed (Boettiger 1960). (ii) The nuclei are irregularly arranged in the fibre. Pringle also says that the fibre diameter of fibrillar types is larger than the close packed types. This is certainly true in some cases (Tachinidae) but in others the fibre diameter is of the same order in both types of muscle.

(e) Lastly there is the rather distinctive muscle of the cicadas (Plate 2.13) which is thought to be histologically intermediate between close packed and fibrillar muscle types and which Pringle has called pseudofibrillar muscle. These pseudofibrillar muscle fibres have peripherally located nuclei and the fibrils, which are of the same dimensions as those of close packed muscle, are arranged in "complex lamellae".

Into which of these five categories may Nudaurelia muscle be placed? Clearly at the outset they are not of type (a) or (b). To decide between the other categories it is necessary first to determine the status of the "cells" visible in the transverse section of the muscle in Plates 2.5 and 2.6. Are these "cells" of 2.0 microns diameter Cohnheim areas or are they large fibrils? The latter alternative is the more likely for two reasons. (i) If the cells were Cohnheim areas it could be expected that in longitudinal section the muscle columns would not retain their individuality throughout the length of a fibre. In fact the limits of the "cells" may be clearly seen running the entire length of the fibre and consequently the "cells" must be fibrils and not Cohnheim areas. (ii) Secondly if the "cells" were Cohnheim areas it could reasonably be expected (especially in fixed tissue) that there would be some indication of fibrils within the Cohnheim areas when seen in transverse section, and within the muscle columns in longitudinal section. In fact there is never any indication of any fibril structure within these "cells" and consequently we must conclude that each of the "cells" must be an individual fibril.

Steedman (1960c) has pointed out that optimally fixed material (approaching sectioned frozen tissue in appearance) may not be the best for revealing the full structure of a tissue and very often the poorly fixed material with much shrinkage is

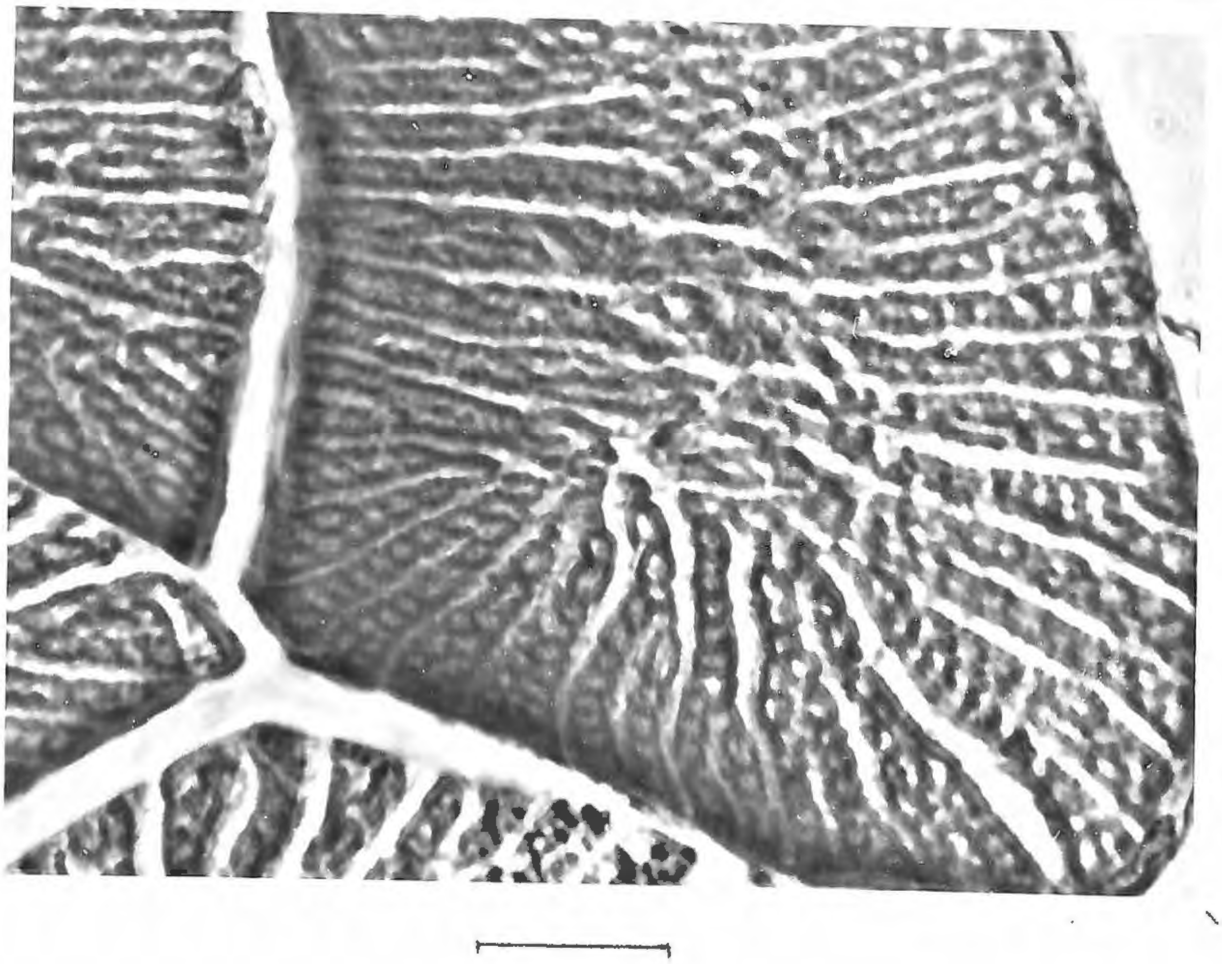


PLATE 2.13. Photomicrograph of a muscle fibre of a Cicada of unknown species, under phase contrast. The dorsal longitudinal muscles (photographed here) and the tymbal muscles are of very much the same appearance. The muscles were fixed in FWA, sectioned at 4 microns, and stained in Heidenhain's Iron Haematoxylin.

more use in this respect.

Consequently before coming to a final conclusion about the status of the flight muscles in Nudaurelia, it is necessary to consider the range of histological appearances that results when the same muscle from the same animal is treated with different fixatives. Plates 2.5 and 2.6 show the excellent fixation obtained with Flemming-without-acetic and it is on these sections that the preceding discussion has been based. Plates 2.7, 2.8, 2.9 and 2.10 show the range of histological appearances that can result from fixation in Alcoholic Bouin - in some cases fibres having these four different appearances may be seen on a single slide depending apparently on the penetrating properties of the fixative. There are two possible ways in which the types of fibre portrayed in Plates 2.7 to 2.10 may be derived from the apparently optimal condition of the fibres fixed in FWA.

(i) It is conceivable that the regions where the fibrils abut in the FWA fixed material, Plates 2.5 and 2.6, have become enlarged, accompanied by shrinkage of the fibrils. It is not hard to imagine that if one of these regions enlarged it would become surrounded by four or possibly five fibrils. If two such enlarging spaces coalesced the resulting space would then the surrounded by approximately eight or nine fibrils. This appears to be the situation in Plate 2.9 where each of the "spaces" are surrounded by approximately five fibrils (very light in colour and demarkated by thin dark strands). A "transitional stage" between the condition in 2.9 and the FWA fixed material is seen in Figure 2.8. If this idea were correct it would be expected that there should be the same number of fibrils per unit area in Plate 2.9 as there are in the FWA material (Plate 2.6). A glance at Plate 2.9 shows that this is not the case - there are in fact approximately five times as many "fibrils" as there should be; but there are approximately the correct number of "black spaces" per unit area to correspond with the fibrils in the FWA fixed material. So the numerical discrepancy rules out this interpretation.

(ii) It is possible to think of this in another way. Suppose the "ground substance" within the fibrils of the FWA fixed material (Plates 2.5 and 2.14) were to shrink - in the less severe cases of shrinkage it might be expected that the "ground substance" would form dark areas in the middle of the fibril and indeed this condition is to be seen in Plates 2.7 and 2.15. As shrinkage proceeds the "ground substance" would become more concentrated in the middle of the fibril leaving minute threads in the resultant space behind it. This could possibly be the condition shown by the material in Plates 2.8 and 2.16. Ultimately the condition shown by the material in Plates 2.9 and 2.17 is reached. (Note that Plate 2.9 is at a higher magnification than any of the others). (Reference to the series of sketches and photographs (Plates 2.14 to 2.17) accompanying each of the Plates 2.5 to 2.9 may assist in the comprehension of this discussion.) The regularity of the minute threads seen in Plate 2.9 is very difficult to explain in terms of this hypothesis and it is also clear that the appearance of the fibres shown in Plates 2.8 and 2.10 are further modified by the cracks in the tissue. Finally it is necessary to attempt to explain the occurrence of the "dark canals" in Plate 2.9 which gives the muscle fibre its lamellar appearance. It would seem that the origin of these dark canals may be traced to the dark regions where the fibrils abut in the FWA fixed material (Plate 2.5). Subsequent swelling, or perhaps shrinkage away from the "dark canals" visible in Plate 2.5 might result in the characteristic lamellar appearance of Plate 2.9.

Perhaps of greatest relevance in this discussion, however, is the resemblance these muscle fibres from <u>Nudaurelia</u> bear to

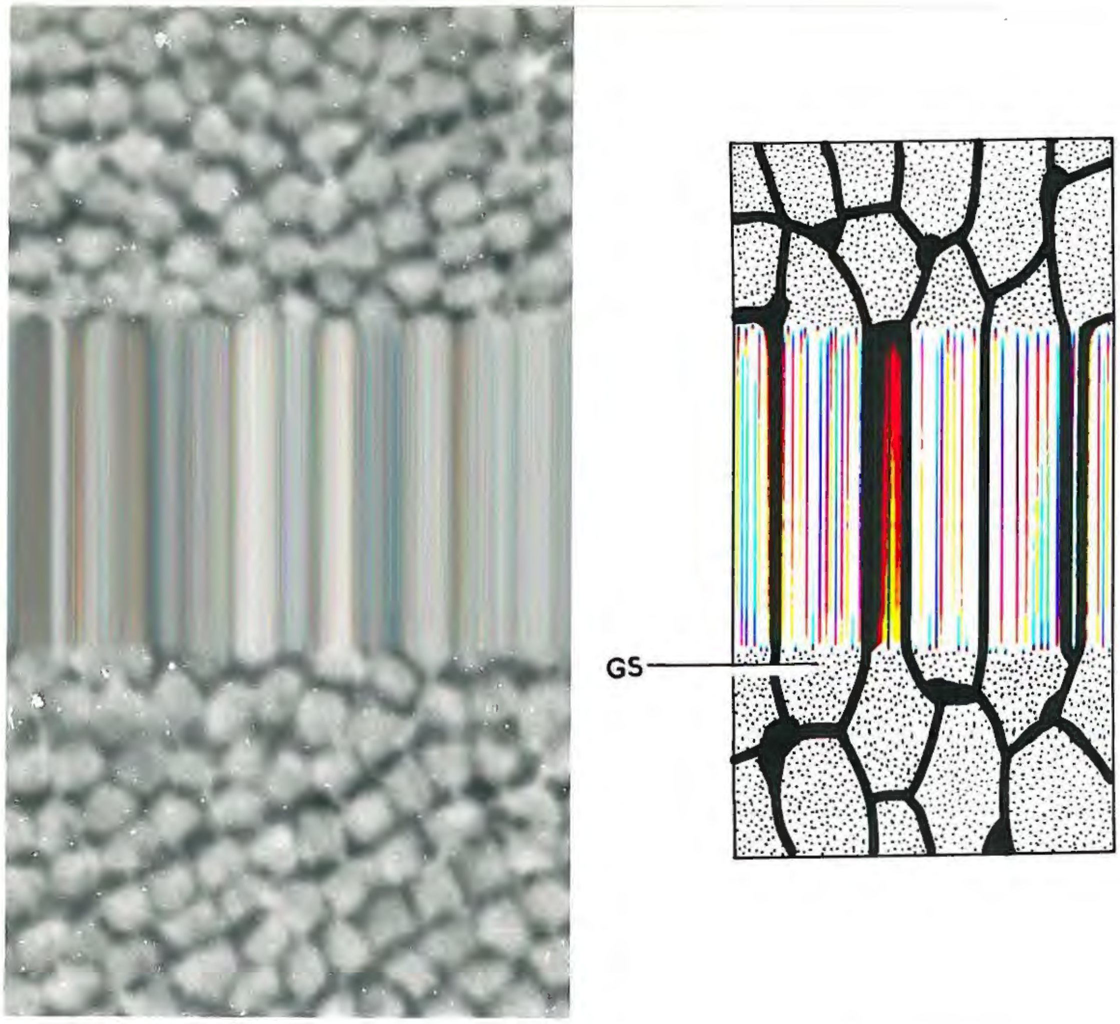


PLATE 2.14. The photomicrograph is an enlarged representative area of the FWA fixed muscle shown in Plate 2.5. The sketch is a diagrammatic representation of the FWA fixed muscle showing the "ground substance" (GS) within the fibrils.

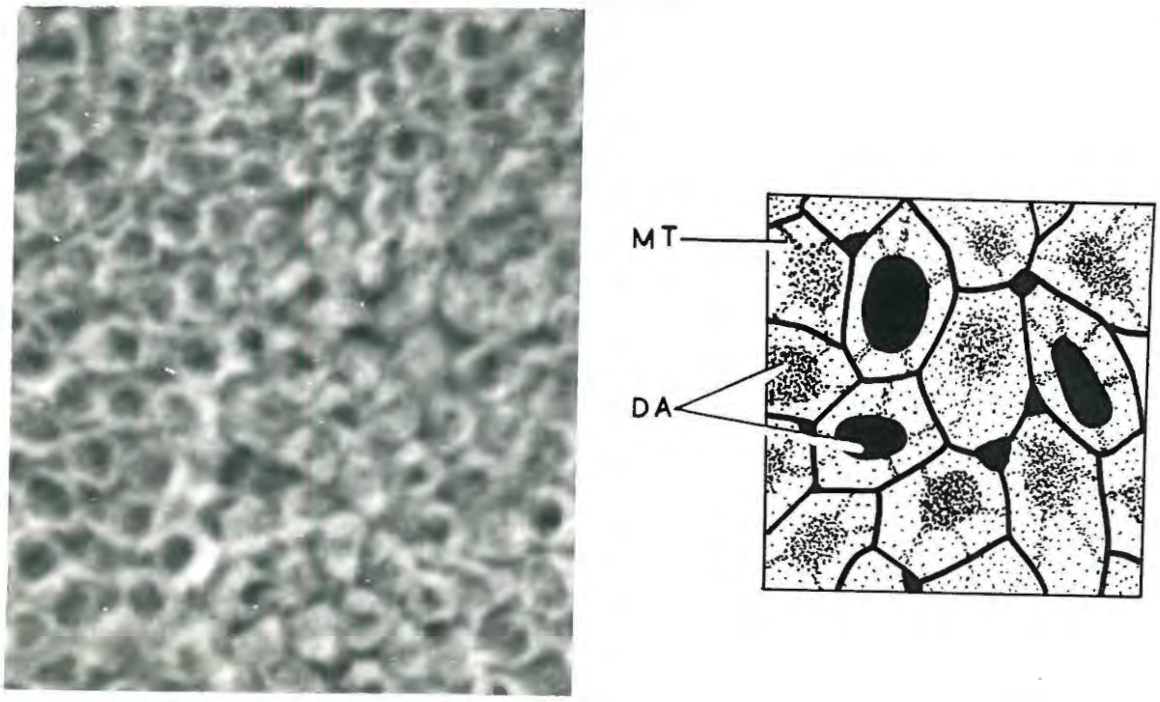


PLATE 2.15. Photomicrograph of an enlarged representative area of muscle shown in Plate 2.7. The sketch is a diagrammatic representation of the photomicrograph showing how the "ground substance" in Plate 2.14 is thought to shrink to the middle of the fibril where it forms "dark areas" (DA) leaving minute threads (MT) in the fibril as it shrinks.

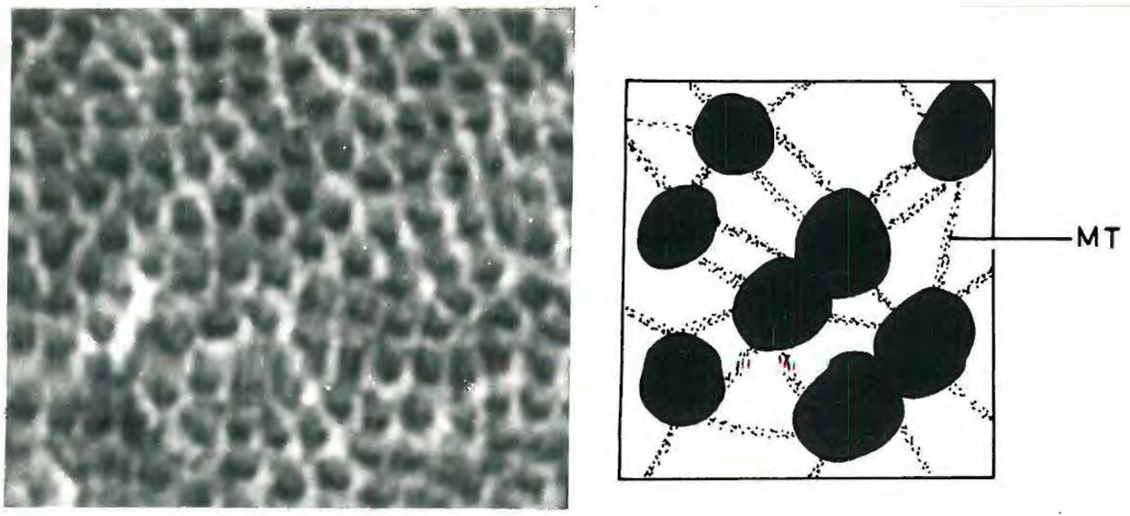


PLATE 2.16. Photomicrograph of an enlarged representative area of muscle shown in Plate 2.8. The sketch is a diagrammatic representation of the photomicrograph showing further shrinkage of the ground substance shown in Plate 2.15. Note the minute threads (MT) around the dark areas of ground substance.

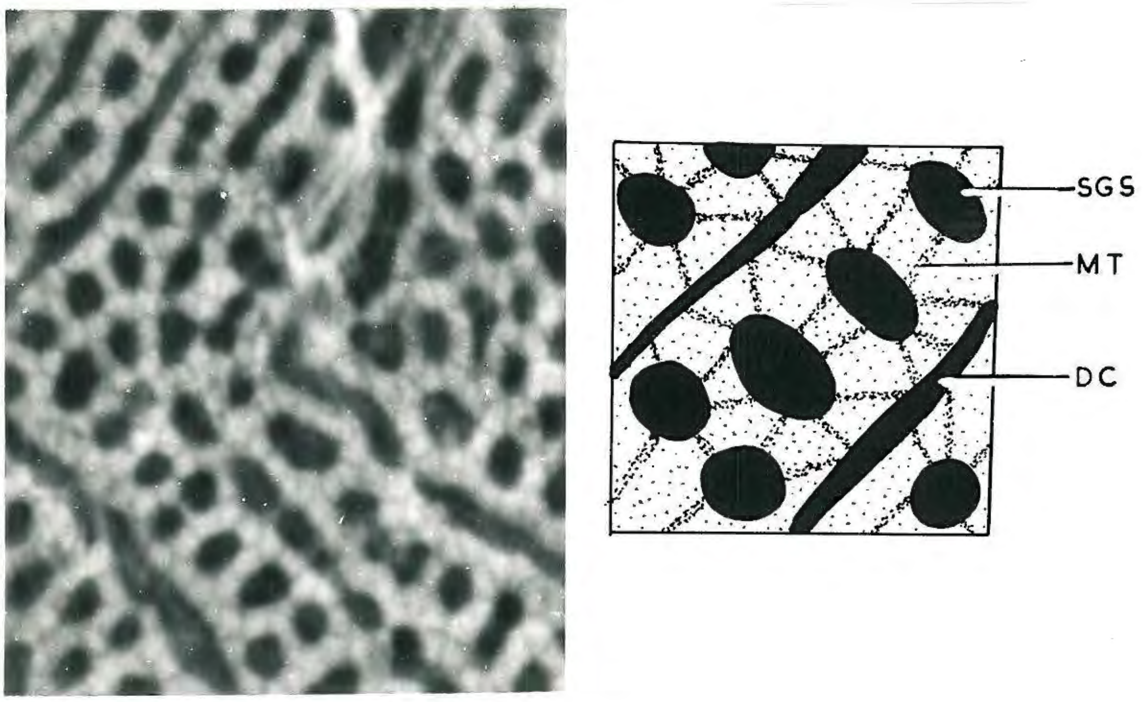


PLATE 2.17. Photomicrograph of an enlarged representative area of muscle shown in Plate 2.9. The sketch is a diagrammatic representation of the photomicrograph showing the shrunken ground substance (SGS) and the minute threads (MT) radiating from it. Note the appearance of the dark canals (DC) which give the muscle its lamellar appearance. For further explanation see text.

some of the histological types figured by Tiegs. The FWA fixed tissue in Plates 2.5 and 2.6 bears a striking resemblance to certain frozen sections of both the fibrillar and non-fibrillar types of muscle illustrated by Tiegs. In particular these sections from *Nudaurelia* look like Tiegs's frozen sections of Honey bee fibrillar muscle. Secondly the appearance of the muscle shown in Plate 2.8 is similar to that of Tiegs's fixed preparations of the rat and the scarabid (*Aphodius howitti*) leg muscle (fixed in alcoholic fixatives and stained with Iron Haematoxylin). Thirdly, and very strikingly, *Nudaurelia* muscles of the appearance in Plates 2.9 and 2.10 look very similar to Tiegs's picture of the pseudofibrillar muscle of the cicadas; further the muscle shown in Plate 2.10, in particular, looks very similar to my own cross-sections of cicada muscle (Plate 2.13) (the cicada muscles have a similar appearance with both alcoholic Bouin and FWA fixation.) Lastly it is totally obscure why the *Nudaurelia* muscles photographed in Plates 2.9 and 2.10 should be so widely wildly different.

Thus with *Nudaurelia* muscles it is possible to obtain histological pictures which are very inconsistent and bear noticeable parallels to some of Tiegs's muscle types. This would seem to suggest that certain of Tiegs's muscle types need reinvestigation to ensure that fixation artifacts cannot account for some of the differences between muscles of different insects.

We are now in a position to consider the histological status of the flight muscles of *Nudaurelia*. The fibrils of *Nudaurelia* are large (2.0 microns and more) and fall within the range specified for fibrils of fibrillar muscle - they are apparently too large for fibrils of the close packed type. However, it is fairly clear that *Nudaurelia* muscles are of the close packed or microfibrillar type as they have peripheral nuclei and respond to direct electrical stimulation unlike those of the fibrillar type. The fibrils are, however, of a particularly large size

One of the most striking features of the dorsal longitudinal muscles is that if any one of the muscle blocks 1 - 5 (Plate 2.2) is stimulated directly it is able to contract alone, and apparently independently of the others. In other words with direct stimulation of a particular muscle block and suitable adjustments of stimulation intensity any particular one muscle block can be made to contract alone and separately from any of the others. This phenomenon was investigated further by directly stimulating each one of the muscle blocks separately at a gradually increasing intensity of stimulation and recording the sequence in which the five muscle blocks contracted. The results of several such experiments are tabulated in Table 2.1.

TABLE 2.1.

Number of muscle block stimulated at 10 shocks/sec.	quence of contraction with gradually increasing stimulation intensity.				
	1st	2nd	3rd	4th	5th
5	5	4	3	2	1
4	4	3	5	2[*]	1[*]
3	3	4	5	2[*]	1[*]
2	4	3	5	2	1
1	4	3	5	1	2[**]

[*] Denotes that these muscles contracted practically simultaneously in the majority of experiments.

[**] Note that in some cases when muscle block 1 is stimulated, muscle blocks 1 and 4 are seen to contract first simultaneously, followed in order by blocks 3, 5 and 2.

A second series of experiments were conducted in which nerve NDL (Plate 2.4) was severed from the ganglion, ligatured, and stimulated at different frequencies and a record kept of the order of contraction of the five muscle blocks. In each case the thresholds of contraction were also recorded. These results are tabulated in Table 2.2.

TABLE 2.2.

Stimulation frequency/sec.	Sequence of contraction of the Muscle Blocks.				
	1st	2nd	3rd	4th	5th
3.25	3^{*}(0.36)	4^{*}(0.36)	5^{*}(0.36)	2^{**}(0.41)	1^{**}(0.41)
6.50	5^{*}(0.31)	4^{*}(0.31)	3 (0.35)	2^{**}(0.45)	1^{**}(0.45)
20.0	3^{*}(0.36)	4^{*}(0.36)	5^{*}(0.36)	2^{**}(0.48)	1^{**}(0.48)
32.0	3^{*}(0.36)	4^{*}(0.36)	5^{*}(0.36)	2^{**}(0.48)	1^{**}(0.48)

* and ** denotes practically simultaneous contraction.

Note the figures in brackets are the thresholds for muscular contraction on stimulation of nerve NDL expressed in volts.

From these two tables it is clear that muscle blocks 3, 4 and 5 and muscle blocks 2 and 1 tend to contract together as functional units. From Table 2.2 it is evident that with stimulation of nerve NDL muscle blocks 2 and 1 have a higher threshold than the other three and a possible explanation of the results in Table 2.1 is that when muscle blocks 2 or 1 are directly stimulated, the electrotonic spread through the haemolymph is sufficient to stimulate muscles 3, 4 and 5 which respond at a lower threshold. However, it may well be that when the electrodes are applied directly to the muscle blocks the nerves within the muscle blocks are themselves being stimulated, and that dromic or antidromic impulses stimulate the other blocks. So it may be that even with the electrodes placed directly on the muscle, the peripheral nerve fibre thresholds are being recorded which may bear little relation to the thresholds for the muscle fibres themselves. In all, however, the data contained in these two tables imply that there are probably two axons involved, the one supplying muscle blocks 1 and 2 while the other supplies muscle blocks 3, 4 and 5. The significance of this implication will become apparent later.

There is one result common to all the preparations which does not seem to aid in any functional analysis but which is worthy of note. This is the phenomenon of maintained tetanus in the flight muscles which continues for some time after stimulation of nerve N2 has ceased. Nerve N2 was ligatured and severed from the ganglion. Nerve N1 and nerve NW were also severed. After the dorsal longitudinal muscles had been stimulated into tetanus by high frequency stimulation of nerve N2 it was noted that tetanus was maintained for varying times after cessation of stimulation depending upon the original stimulation frequency. These results are presented in Table 2.3.

TABLE 2.3.

Frequency of Stimulation per second.	Duration of tetanus maintenance after cessation of stimulation.
350	2 - 3 seconds.
90	4 - 5 seconds
65	10 - 12 seconds
35	Approximately 30 seconds.

At all frequencies, tetanus was maintained for five seconds after which stimulation was stopped, and throughout the experiments the stimulus output from the stimulator was monitored on an oscilloscope and consequently the results cannot be accounted for by some defect in the stimulating apparatus. There would appear to be no immediate explanation of these results.

(b) Secondly, recordings made of the electrical activity of the flight muscles during flight remain to be considered.

As I have already mentioned and as Pringle (1957) has emphasised, the flight muscles of insects fall into two distinct physiological categories - the more primitive condition in which there is a 1 : 1 relationship between the number of action potentials recorded from the flight muscles and the wing beat frequency, and the physiologically more specialised insects in which the wing beat frequency exceeds the number of action potentials recorded; that is, the ratio is greater than unity. The term synchronous flight has been used (McCann and Boettiger 1961, and others) for the former condition and asynchronous for the latter.

Recordings from the dorsal longitudinal flight muscles of Nudaurelia, and the hawk moth Theretra capensis, during flight and during warm-up, were made with the following results.

Recording from Nudaurelia during normal flight it was evident that flight was synchronous and that the moth flaps its wings at a frequency of about 10 per second. Surprisingly, however, there were found to be 4 - 7 action potentials per wing beat (see Trace 2.A); this situation, where there is more than one action potential per wing beat, does not seem to have been recorded for any other insect. Thus Roeder (1951) has recorded one action potential for each wing beat from the dorsal longitudinal flight muscles in Periplaneta and Agrotis. Baranowski (unpublished - quoted in Pringle 1957) has also recorded a 1 : 1 relationship between muscle action and wing beat frequency in a sphingid. I have found there to be one action potential per wing beat in an unidentified Cicada. The remote possibility exists that some of the action potentials seen on Trace 2.A might be related to the contraction of the direct wing muscles, the action potentials being carried to the electrodes by electrotonic spread. This is unlikely for three reasons:-

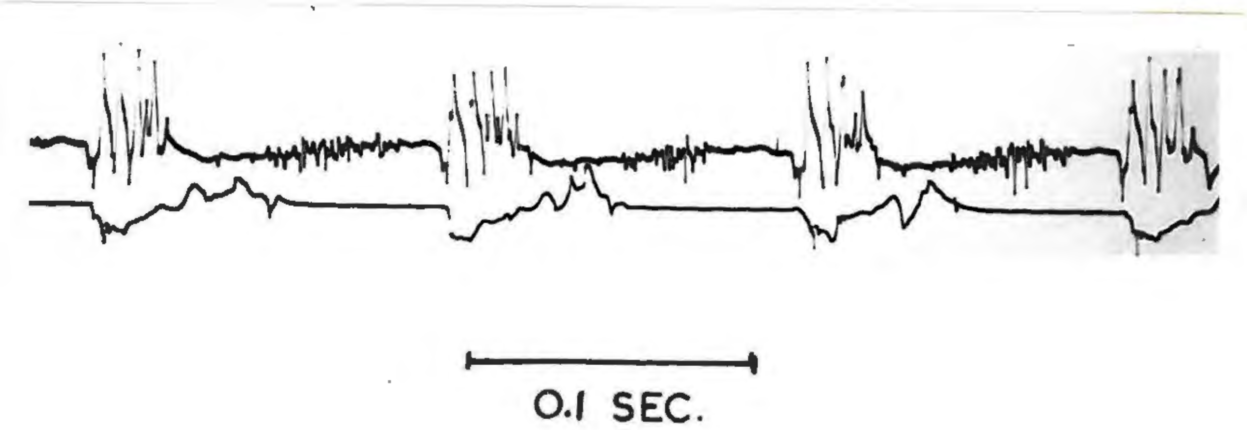
(a) The electrodes were inserted directly into the dorsal longitudinal muscles and were about 3 - 4 mm. away from the nearest direct wing muscle.

(b) The action potentials from the tergo-sternal muscles, which are much larger than the direct wing muscles and were in much closer proximity to the electrodes, appear as rather small potentials between the large action potentials from the dorsal longitudinal muscles (Trace 2.A). Thus it is improbable that action potentials from the direct muscles would be visible.

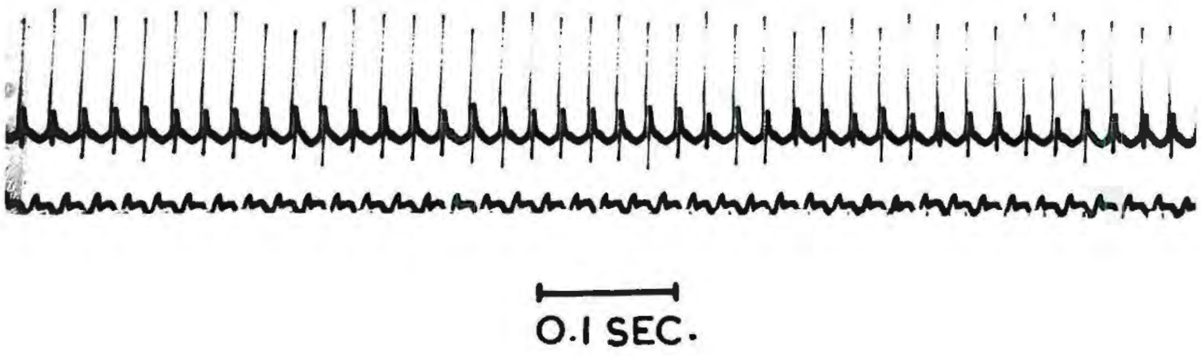
(c) If in fact action potentials from the direct wing muscles were leaking through to the recording electrodes in Nudaurelia it could be expected that there would be a similar effect when recording from the dorsal longitudinal muscles of the hawk moth. In the latter case there is clearly one action potential from the dorsal longitudinal muscles per wing beat and no indication of any potentials from any of the direct wing muscles (Trace 2.B).

It is possible that the significance of the condition in Nudaurelia where there are several action potentials per wing beat may be related to the need for a powerful and yet even contraction throughout the "power" or downstroke of the wing beat. In other words with such large broad wings it would seem functionally very inefficient and perhaps impossible to contract all five longitudinal muscle bundles simultaneously at the beginning of the downstroke. The rational procedure functionally would be to contract the muscle blocks in sequence through one downstroke and so maintain an even contraction during the stroke. Three lines of evidence may be considered in relation to this thesis.

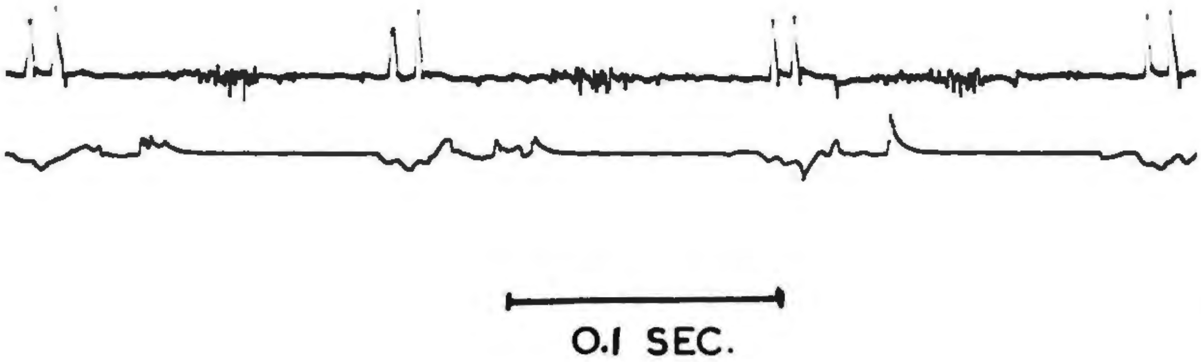
(i) It is evident from the results already recorded that, with artificial stimulation, the five dorsal longitudinal muscle blocks can and do contract independently of each other and that muscles 1 and 2 which seem to be responsible for the last part of the downstroke have a higher threshold than the other muscle blocks irrespective of whether they are stimulated directly or by way of nerve N2. However, it cannot be argued that because



TRACE 2.A. Oscillograph records from the dorsal longitudinal muscles of Nudaurelia recorded during normal tethered flight. The upper trace is the record of action potentials from the muscles, and the lower trace is a record of the thoracic distortion. The irregular portion of the lower trace represents the duration of the downstroke, while the straight horizontal portion represents the downstroke. Note several action potentials for each downstroke and the presence of several, much smaller, action potentials during the upstroke.



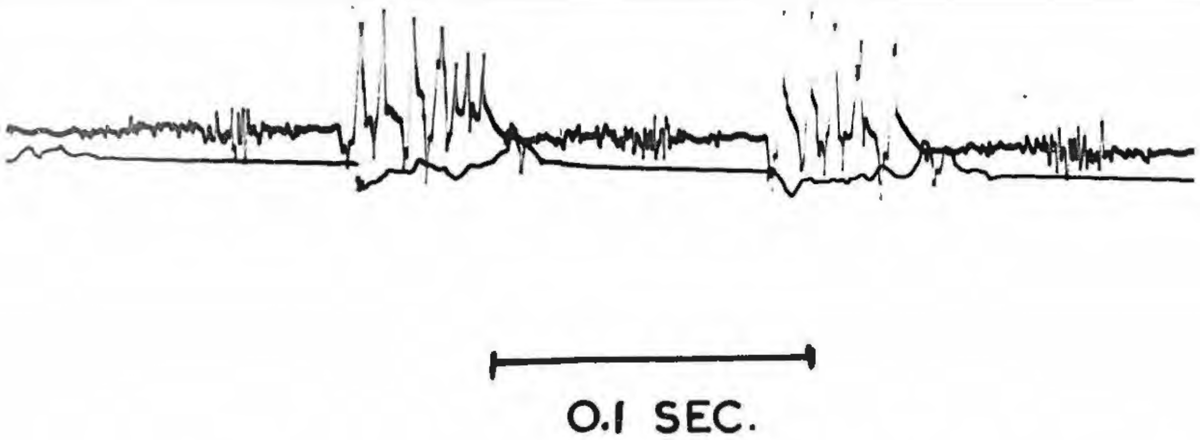
TRACE 2.B. Oscillograph record from the dorsal longitudinal muscles of Theretra during normal flight. The upper trace is the record of the action potentials from the muscles and the lower trace is a record of the thoracic distortion. Note one action potential per wing beat stroke.



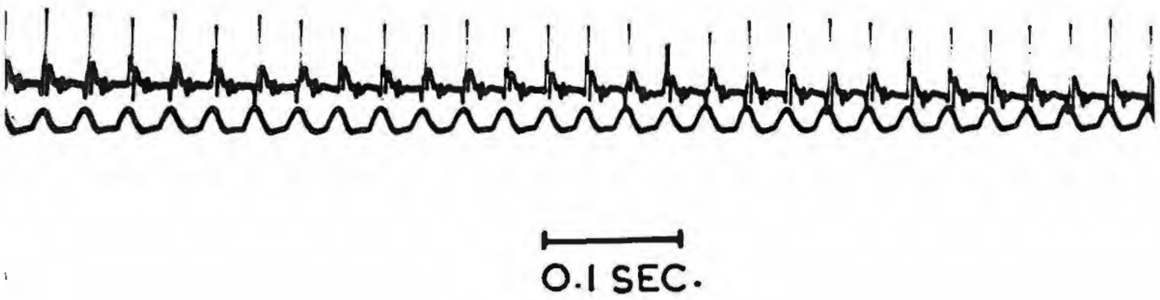
TRACE 2.C. Oscillograph record from the dorsal longitudinal muscles of Nudaurelia during "weak" flight. Legend as for Trace 2.A except for the presence of only two action potentials per wing beat stroke. (See text for further explanation).

the nerve fibres have a high peripheral threshold, they also have a high central threshold. So there is no reason to believe that because the muscles contract independently and in a certain order, with peripheral stimulation, that this is the program relayed to the muscles from the central nervous system during normal flight. In point of fact evidence presented later would seem to substantiate this doubt.

(ii) In emperor moths when the wings are beating strongly there are 5 - 7 action potentials per wing beat (Trace 2.A). However, on some occasions the flapping becomes noticeably weaker and the arc traversed during the beat is very much decreased. During this "weak" flight the wing beat frequency does not decrease but the number of action potentials per wing beat is reduced from 5 to 2 (see Trace 2.C). This would seem to suggest that only two muscle bundles are operative during weak flight and that the power of the beat is increased when the other three muscles are activated. If then we believe that during the downstroke there is one action potential related to the contraction of each of the five muscle blocks, why are there in fact normally 7 action potentials per downstroke? The solution to this becomes clearer when muscle block 5 is examined more closely as it is to be seen that this muscle block is in fact divided up into three smaller parts (see Plate 2.3). Consequently it could be expected that there would be 7 action potentials per wing beat and that there should be three small action potentials and four larger action potentials. This situation is not always apparent but is true for many of the recordings (see Trace 2.D). However, at this point there is an important flaw in the argument. If, as has been suggested up to now, the muscle blocks were contracting in sequence from the dorsal side ventrally, the three small action potentials, in Trace 2.D, should precede and not follow the four larger action potentials. These traces seem to indicate therefore that the sequence of contraction of the muscle blocks during normal flight is ventro-



TRACE 2.D. Oscillograph record from the dorsal longitudinal muscles of Nudaurelia recorded during normal flight to show one of the traces in which it is possible to see four large action potentials followed by three smaller action potentials (See text for explanation). For the remainder of the legend see Trace 2.A.



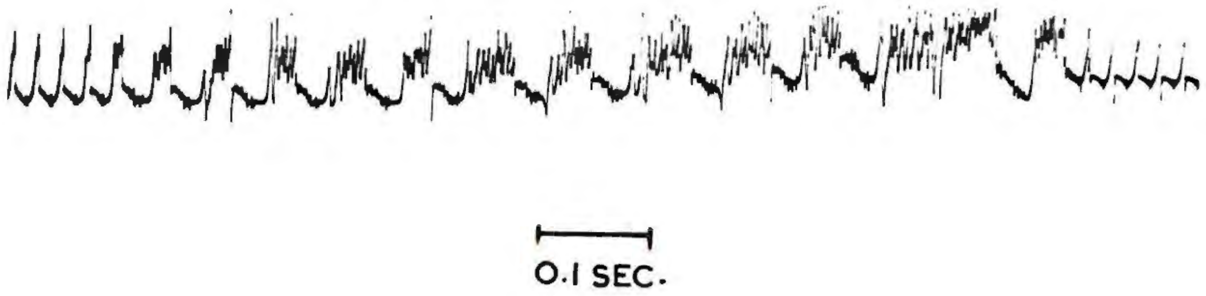
TRACE 2.E. Oscillograph record from the dorsal longitudinal flight muscles of Theretra during "weak" flight. For the remainder of the legend see Trace 2.B.

dorsal, and is further indication that the results obtained by peripheral stimulation and the actual central flight program are at variance. In any event there does appear to be an obvious explanation in mechanical terms of the possible advantage of a sequential ventro-dorsal muscle contraction over a contraction in sequence dorso-ventrally. In essence the dorsal longitudinal flight muscles are required to buckle the tergum of the thorax dorsally and so produce the downstroke of the wing beat. By contracting the muscles in sequence vento-dorsally it is clear that the most ventral muscle, which is the most distal from the tergum, will have the maximum leverage and be able to initiate the downstroke with the minimal effort. Once this inertia has been overcome it is easier for the more dorsal muscles to contract and increase the velocity of the downstroke.

(iii) Further evidence in favour of the theory that a contraction of each of five muscle blocks in sequence is a device to give extra power in conjunction with a smooth contraction is gained from a study of hawk moths. Hawk moths also may display both normal and weak flight. In this case, however, there is a decrease in wing beat frequency during weak flight and but one action potential per wing beat although its amplitude is smaller than the normal (see Trace 2.E). If a hawk moth is maltreated in some way during normal flight (the antennae are pinched or the abdomen squeezed) flight will cease temporarily and the animal gives a series of slow, extremely powerful flaps which traverse an arc far larger than that during normal flight and equal to that of normal flight of Nudaurelia. This phenomenon may be called "forced flapping". The frequency of forced flapping is approximately 11 per second (very nearly equal to normal flight frequency in the emperor moth) and during forced flapping several action potentials are recorded from the dorsal longitudinal muscles for each downstroke. As normal flight is resumed the condition of one action potential per beat is restored - this point is illustrated in Traces 2.F and 2.G.

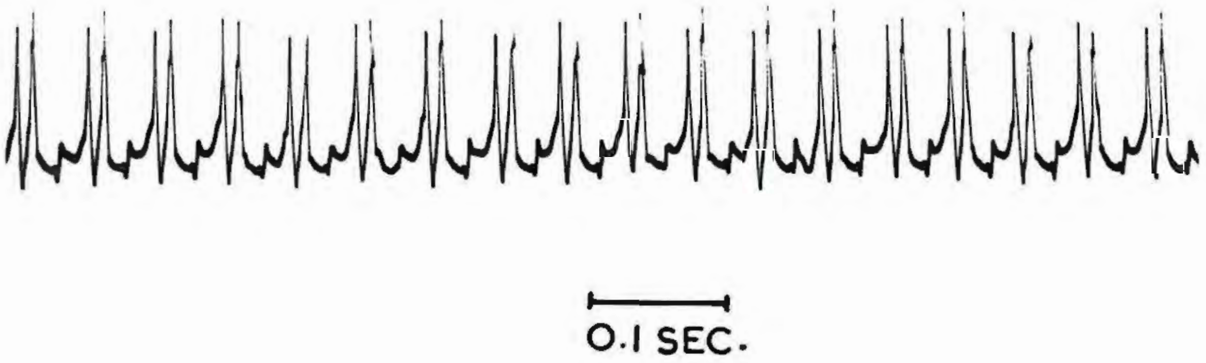
Finally it must be mentioned that attempts were made to verify this hypothesis by recording from only one of the muscle bundles in Nudaurelia in the hopes that only one action potential would be recorded and that it would be possible to relate this action potential to a particular phase of the cycle of the down beat. Unfortunately these attempts were unsuccessful in all cases. Even when only the very tips of the electrodes were left uninsulated several action potentials were recorded per wing beat. It seems likely that electrotonic spread accounts for the failure of these experiments - it is known that when recordings are made from the dorsal longitudinal muscles the electrodes pick up potentials from the dorso-ventral muscles although these muscles are relatively remote to the recording site (see Trace 2.A),

What of the action potentials recorded from the dorsal longitudinal muscles, in these two species of moth during warm-up? Warm-up in both species is a very clearly defined phenomenon and very distinct from flight movements. "Warm-up" is a period during which the wings vibrate through an arc far less than that found during normal flight. The frequency of beat during warm-up in Nudaurelia is about twice that of free flight and the arc traversed by the wing tips during warm-up, i.e. the amplitude of the stroke, is very small and usually only a few mm. During flight the distance traversed by the wing tips is at least 10 cm. in Nudaurelia and about 3 cm. in Theretra. This warm-up (which Pringle describes as being a period of "wing-whirring") may also occur between two periods of strong flight. Thus warm-up and flight movements are very easily distinguished and are never confused with each other. During warm-up in the emperor moth the wings oscillate at a frequency of about twenty per second and there are two action potentials per wing beat (see Trace 2.H). During warm-up in hawk moths the wings oscillate at about forty

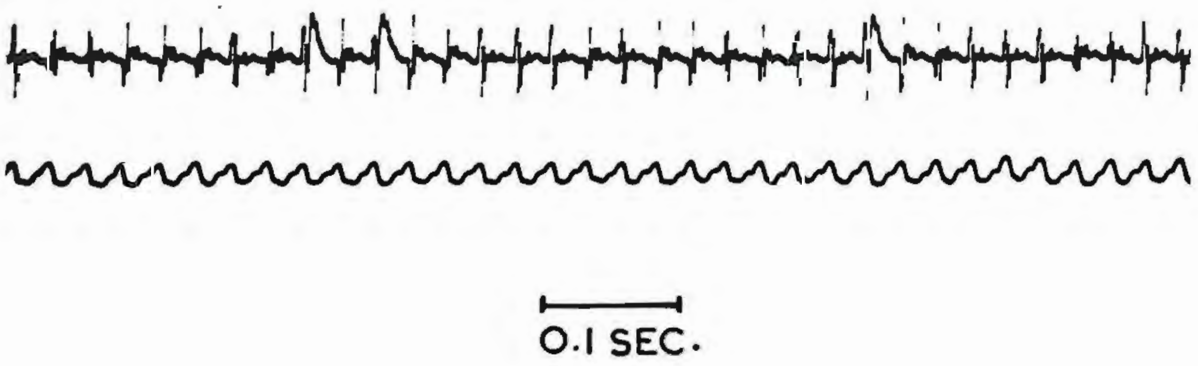


TRACE 2.F. Oscillograph record from the dorsal longitudinal muscles of *Theretra* showing normal flight action potentials (at either end of the record) interrupted by a series of action potentials from "forced flaps".

TRACE 2.G. Legend as for Trace 2.F.



TRACE 2.H. Oscillograph record from the dorsal longitudinal muscles of Nudaurelia during "warm-up" showing the presence of two action potentials for each warm-up "stroke".



TRACE 2.I. Oscillograph record from the dorsal longitudinal muscle of Theretra during "warm-up". Note the presence of one action potential for each warm-up stroke. The upper trace is the record of action potentials and the lower trace the record of the thoracic distortion.

beats per second and there is only one action potential per wing beat (Trace 2.I).

Some of the data contained in this section are summarized in Table 2.4. The implications of these results are considered at greater length in the discussion.

TABLE 2.4.

Animal.	Type of wing beat.	Wing beat frequency per second.	Action potentials per wing beat.
Nudaurelia	Normal flight	7 - 11	4 - 7* : 1
Theretra	Forced flapping	11 - 14	5 + : 1
Nudaurelia	Weak flight	7 - 11	2 : 1
Nudaurelia	Warm-up	20	2 : 1
Periplaneta	Normal flight	30	1 : 1
Theretra	Warm-up	37	1 : 1
Theretra	Weak flight	32 - 44	1 : 1
Theretra	Normal flight	48 - 55	1 : 1
Diptera	Normal flight	More than 150	1 : 9 - 18

* Note. In very rare cases there are as many as 8 or 9 action potentials per wing beat. No explanation can be offered. (See Trace 2.J).

(iii) The Central Nervous Basis of Warm-Up in Nudaurelia.

Until recently the coordination of flight in insects has been accounted for in terms of simple reflex controls (Weis-Fogh 1956 and Pringle 1957). Wilson (1961), however, presents evidence to support the hypothesis that there is an innate flight pattern built into the central nervous system which, even in the absence of any sensory input, is sufficient to produce flight movements in the locust. Wilson points out that the central nervous program can, and is, modified by sensory feedback and that the peripheral sensory loops might themselves be sufficient to produce flight movements, but still he visualizes the central nervous flight program as being of primary importance. As far as is known the central nervous basis of "warm-up" in insects has never before been investigated and it seemed worthwhile to examine the phenomenon of "warm-up" in Nudaurelia in the light of Wilson's results.

Warm-up is distinct from normal flight in two important respects - (a) the amplitude of the wing beat is very small, and (b) in some cases (in Nudaurelia at least but not in Theretra - See Table 2.4), the oscillation frequency of the wings is increased. It seems likely therefore that the "warm-up" programs and flight programs from the central nervous system are different. From this the question arises as to whether the central "warm-up" program is a reorganization of the normal flight program or whether there is a distinct centre responsible for the warm-up program. The sensory aspects involved are not considered in this investigation.

By reference to Plate 2.4 it is evident that the likely location for the "warm-up" centre must be either in the prothoracic ganglion or in the combined mesothoracic and metathoracic ganglia (which will be referred to as the pterothoracic ganglion in future). If the warm-up centre is located in the pterothoracic ganglion the likely axonal pathways are through nerve N2 and nerve NDL to the longitudinal flight muscles and through nerve NDV and two other nerves, not illustrated in the diagram, to the dorso-ventral flight muscles. On the other hand the warm-up centre may be located in the prothoracic ganglion and the nervous pathways may either run through nerve N1 which joins nerve N2 and innervates the dorsal longitudinal muscles, or they may run from the prothoracic ganglion through the interganglionic connectives by way of nerve N2 to the dorsal longitudinal muscles. Also if the warm-up centre is located in the prothoracic ganglion the dorso-ventral muscles must receive axons by way of the interganglionic connectives and the anterior portion of the pterothoracic ganglion. A more unlikely theory remains to be considered and that is that the warm-up centre may be located in the pterothoracic ganglion with axonal connectives passing through the interganglionic connective IGC to N1 and from there to the dorsal longitudinal muscles. In this case the dorso-ventral muscles would be innervated directly through nerve NDV and others.

The following observations and experiments indicate which of these alternatives is the more likely.

Initially it was noted that if, during vivisection, the prothoracic ganglion was touched with a dissecting instrument, the wing muscles could be made to shiver in a manner very similar to warm-up. Touching the pterothoracic ganglion produced violent flapping. This suggested that the warm-up centre might be located in the prothoracic ganglion. Direct current stimulation of the prothoracic ganglion with the wing flapping freely (for details see Material and Methods), resulted in apparent warm-up which backs up the last observation. Direct current stimulation of the pterothoracic ganglion and more intense stimulation of the prothoracic ganglion produces flapping but warm-up could not be induced by stimulation of the pterothoracic ganglion. Intense stimulation of the pterothoracic ganglion results in tetanic contraction of both sets of wing muscles. The muscles contract tetanically with the wings in the up or down position depending on the polarity of the stimulating electrodes. What determines whether the wings will flap to the up or down position (as both dorsal longitudinal and tergo-sternal muscles seem to contract tetanically) is not clear.

The possibility that the warm-up centre may be located in the prothoracic ganglion was verified in the following experiments in which certain nerves were severed and the effect on the dorsal longitudinal muscles, and on the movement of the wings, were noted.

(a) In the almost hemisected preparation after warm-up has commenced severing the interganglionic connective IGC close to the prothoracic ganglion i.e. anterior to the point of entry of N1, immediately stops warm-up. Flapping may still be induced by stimulating the pterothoracic ganglion although this flapping cannot be maintained and appears to be less powerful than when the connectives are intact. This might imply that there is some sensory input entering the prothoracic ganglion (possibly from the head) which is modifying in some way the flight program in the pterothoracic ganglion. This is in accordance with Wilson's (1961) observations where he shows that peripheral sensory routes influence the central program patterning. Warm-up cannot be initiated at all.

(b) Secondly cutting nerve N2 does not prevent warm-up. This operation does, however, prevent any consistent flapping although occasional flaps are still possible, presumably brought about by the dorso-ventral muscles and occasional twitches from the dorsal longitudinal muscles.

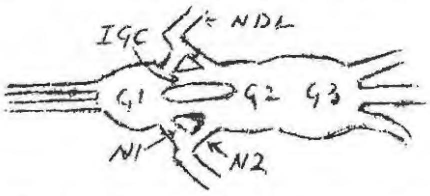
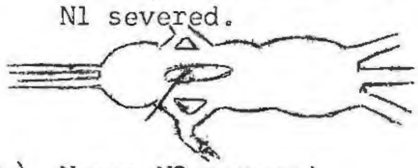
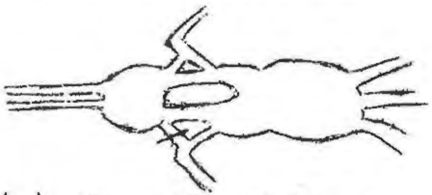
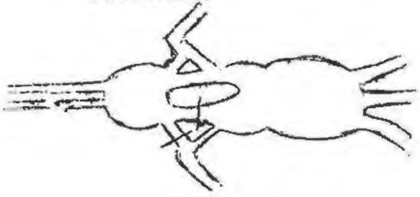
(c) Thirdly severing the interganglionic connectives at the level of the pterothoracic ganglion and cutting nerve N2 while nerve N1 was left intact had the effect of completely preventing any suggestion of flapping. Warm-up was still possible, however, although somewhat weaker than normal and was probably due to the action of the dorsal longitudinal muscles.

(d) Fourthly an experiment was conducted in which nerve N1 was cut while the other nerves were left intact. As could be expected from the previous experiments this did not affect flapping significantly. Weak warm-up was still possible although it was clear that the dorsal longitudinal muscles were not assisting in this weak warm-up and that it was due to the dorso-ventral muscles.

(e) Finally nerve N1 was severed in addition to the interganglionic connectives close to the prothoracic ganglion and nerve N2 was left intact. After this operation warm-up could never be reintroduced but isolated flapping was still possible.

The results of these experiments are tabulated in Table 2.5.

TABLE 2.5.

OPERATION.	EFFECT.
Control - all nerves intact (for legend see Plate 2.4)  	Normal. Moth can be made to warm-up and flap strongly for some time.
(a) Interganglionic connectives (IGC) anterior to N1 severed. 	Warm-up ceases and cannot be re-initiated. Weak isolated flaps still possible.
(b) Nerve N2 severed. 	Warm-up still possible. Bursts of flapping prevented although single occasional flaps do occur.
(c) Nerve N2 and interganglionic connectives severed. 	All flapping prevented. Warm-up not prevented but is weaker than normal only dorsal longitudinal muscles involved?
(d) Nerve N1 severed. 	Flapping apparently unaffected. Very weak warm-up shiver still possible involving the dorso-ventral muscles and not the dorsal longitudinal muscles?
(e) Nerve N1 and interganglionic connectives severed. 	Warm-up completely prevented. Isolated flapping still possible.

NOTE: Experiments which were planned involving the severing of the nerves supplying the dorso-ventral muscles were left until last as practically the experiments are very difficult. The adult population, however, died before any experiments were completed.

From the simple experiments summarised in Table 2.5 it is possible to come to some conclusions about the location of a "warm-up" centre in Nudaurelia. It can be stated with some certainty that the "warm-up" centre is not located anterior to the prothoracic ganglion because decapitation does not prevent "warm-up". This was confirmed in several animals. Secondly if the interganglionic connectives are severed, anterior to nerve N1, (operation (a)) warm-up ceases completely. This must mean that the warm-up centre is located in the prothoracic and not in the pterothoracic ganglion. If nerve N2 is severed warm-up is unaffected (operation (b)). Where the interganglionic connectives IGC are severed behind nerve N1 and nerve N2 is also cut, warm-up is impaired and apparently only involves the dorsal longitudinal muscles. Thus there seems to be an axon from the warm-up centre running via nerve N1 and supplying the dorsal longitudinal muscles. Operation (d) shows the effect of severing nerve N1 while leaving the interganglionic connectives intact - warm-up is again impaired and in this case seems to involve only the tergo-sternal muscles. This suggests an axon running from the warm-up centre through the interganglionic connectives to the pterothorax and from there via nerves NDV to the tergo-sternal muscles. In the final experiment (operation (e)) in which the interganglionic connectives and nerve N1 are severed these suppositions are confirmed as all warm-up is prevented.

Thus it is visualized that axons from the prothoracic warm-up "centre" supply the dorsal longitudinal muscles by way of nerve N1 and not via nerve N2, and that the dorso-ventral flight muscles are innervated by axons from the warm-up centre via the interganglionic connectives and the mesothoracic ganglion.

Lastly it is noteworthy that of the dorsal longitudinal muscles of Nudaurelia, muscle blocks 1 and 2 seem to be the main muscles involved in producing warm-up. This cannot be stated finally as it is extremely difficult to see precisely which muscles are involved when the wings and the thorax are shivering violently during warm-up. This observation, if true, solves one problem and poses another. If muscles 1 and 2 are the only longitudinal muscles involved in warm-up it would explain why there are only two action potentials per warm-up stroke (see Trace 2.H). It is tempting to imply from this that muscles 1 and 2 are responsible for the onset of the downstroke and this would seem to substantiate the idea gained from Trace 2.D. In addition this might explain the differences in peripheral threshold between the muscle blocks 3, 4, 5 and 2, 1 obtained with stimulation of nerve NDL. What is peculiar, however, is that Nuesch (quoted in Pringle 1957) shows, in his studies on Telea, that muscle blocks 1, 2, 3 and 4 are all innervated from the prothoracic ganglion via nerve N1 and it is hard to explain why, if the warm-up centre is in the prothorax, the muscle blocks 1 - 4 are not all involved in warm-up. But there is the possibility that Nudaurelia may differ, in details of innervation, from Telea.

These results also contain implications about the location of a "flight centre" and the innervation of the dorsal longitudinal muscles of Nudaurelia which will be considered in the discussion.

DISCUSSION.

Several workers (Cremer 1934, Tiegs 1955, Buchthal, Weis-Fogh and Rosenfalck 1957, Pringle 1957) have commented on the fact that, in insects that possess non-fibrillar flight muscle action potential per wing beat, reflecting the contraction of one muscle bundle and not the whole dorsal longitudinal muscle.

This simple hypothesis would account for the recorded wing beat frequencies of all those insects with non-fibrillar flight muscle and with synchronous flight. It offers an alternative explanation to the suggestion forwarded by Buchthal, Weis-Fogh and Rosenfalck (1957). They point out that during flight in Schistocerca there is an 8°C rise in temperature in the thorax (Sotavalta 1954 as quoted in Pringle 1957 has shown that warming up movements increase the thoracic temperatures of several insects). This increase in temperature has the effect of decreasing the twitch duration of the muscles and this effectively increases the frequency at which the muscles will go into clonus. This explanation may certainly hold for Schistocerca but, Theretra can be induced to fly at a temperature of ca. 17°C without preliminary warm-up. However, there was no way of estimating the wing beat frequency in these conditions as the animal was flying freely in the laboratory, and the frequency might well be far below that for the normal wing beat.

It is also well known that the actual cuticle of the thorax has elastic properties which affect the duration of the muscle twitch. To what extent this factor may offer a solution to the problem in Theretra is unknown. It must be emphasised that the achievements of the asynchronous very high frequency fliers fall into a completely different category and the problem of high frequency wing beats in these cases has previously been adequately explained.

Suppose again that the theory of sequential contraction of muscle blocks during the wing beat of Nudaurelia is acceptable, the problem arises as to the possible nervous mechanisms that would be required to control these contractions. There are two alternatives (i) It could be that the conduction time along the nerve NDL supplying the dorsal longitudinal muscles is sufficient-slow to produce the required delay between the contraction of each of the muscle blocks. Measurements were made of the delay between each of the five muscle action potentials recorded from the dorsal longitudinal muscles of Nudaurelia during a downstroke and this delay was found to vary between 9 and 13 milliseconds (average 11 msec.). The approximate distance across one of the dorsal longitudinal muscle blocks is 1.3 mm. and thus, if this theory were to hold good, the conduction time along the nerve NDL would be 1.3 mm./ 11 msec. or, in other words, 0.121 metres/sec. Pumphrey and Rawdon-Smith (1937) and Roeder (1948) report conduction velocities of 6 - 7 metres/sec. in Periplaneta abdominal nerve cord giant axons, and velocities of 2 - 3 metres/sec. in the cercal nerve of the same animal. There is no other information on conduction velocities in insect nerves but these data are sufficient to indicate that the conduction velocities along nerve NDL would have to be absurdly low if they were to account for the delay between the contractions of the muscle blocks. This alternative is thus ruled out. (ii) The second alternative would be in terms of a central nervous "centre" or "centres" bringing about the sequential contraction of the muscle blocks. In this case what evidence is there about the location and functioning of the flight centre and the innervation of the dorsal longitudinal muscles from the flight centre in Nudaurelia? Firstly it is evident, in Nudaurelia, that the prothoracic ganglion is concerned in some way with the production of flight movements. If the interganglionic connectives between the pterothorax and prothorax are severed flight movements are very seriously impaired and the animal is only able to produce isolated flaps. Wilson (1961) reports that in isolated cases Schistocerca is able to beat its wings strongly when the nervous connectives anterior to the pterothorax are severed. Chadwick (1953), however, reports that Periplaneta is unable to beat its wings if the interganglionic connectives are cut just posterior to the prothoracic ganglion. This suggests in Nudaurelia and Periplaneta, at least, that a flight centre is located in the prothoracic ganglion and in additon Wilson does state that "The main flight control pattern in Schistocerca is established in the pterothorax with possibly some necessity for the prothoracic ganglion".

Further, the fact that Nudaurelia is able to produce isolated flaps when the prothoracic ganglion is severed from the pterothoracic ganglion supports the idea that there might be a second flight centre located in the pterothorax. The results of peripheral stimulation of nerve NDL to the dorsal longitudinal muscles of Nudaurelia, have suggested that the muscle blocks 1 and 2 are separately innervated from muscle blocks 3, 4 and 5; muscle blocks 1 and 2 are innervated via nerve N1 from the prothoracic ganglion - muscle blocks 3, 4 and 5 receive innervation from a centre in the pterothoracic ganglion. It is satisfactory to note that Nuesch (in Pringle 1957) has recorded a neural connection between the prothoracic ganglion and the dorsal longitudinal muscles by way of nerve N1, and from the pterothoracic ganglion via nerve NDL to the dorsal longitudinal muscles.

From this a tentative account of the flight centres in Nudaurelia can be put forward. There is a prothoracic pacemaker or centre which innervates and initiates contraction in muscle blocks 1 and 2 at the beginning of the downstroke. The prothoracic centre also relays to the pterothoracic flight centre via the interganglionic connectives and the latter centre is then responsible for activating muscle blocks 3, 4 and 5 in sequence to complete the downstroke.

The situation as far as the tergo-sternal muscles is concerned is obscure but presumably they are also controlled by one centre in the prothoracic ganglion and another in the pterothoracic ganglion.

Finally (a) it is evident that if the sequential contraction of the five muscle blocks is the result of a central nervous programming of pattern from the centres, it should follow that there would be some consistency in the pattern and amplitudes of the spikes recorded from the dorsal longitudinal muscles during flight. On the contrary, however, the patterns and amplitudes of the spikes recorded from Nudaurelia and Theretra during flight (Traces 2.A and 2.B) do not show any consistency. (b) Secondly the evidence presented suggests that there are only two axons involved in the innervation of the dorsal longitudinal muscles of Nudaurelia and this number of axons would be quite inadequate to bring about the sequential contractions of five muscle blocks in response to a complex program from the central nervous system.

These facts, then, cast serious doubts on any theory in favour of a sequential contraction of muscle blocks, and consequently another more plausible theory remains to be advanced to account for the presence of several action potentials per wing beat recorded from Nudaurelia.

It seems likely that the flight centres in the central nervous system are emitting volleys of pulses associated with each wing beat. The frequencies of the pulses within the volley are rapid enough (ca. 100 per second) to tetanize the dorsal longitudinal muscles and thus it is possible to imagine that the volley of impulses is concerned with maintaining a tetanus for the whole duration of the downstroke. In Nudaurelia the duration of the stroke is particularly long and might well necessitate a whole volley of pulses to maintain tetanus throughout the stroke. In forms such as Theretra the duration of the stroke is so short that one single nerve spike will activate the dorsal longitudinal muscles for a sufficient length of time. Possibly the phenomenon of weak flight in *Nudaurelia*, where there are only two or three action potentials per wing beat, could be explained in terms of this hypothesis; the reduced number of action potentials producing a less powerful and shorter tetanic contraction. By the same token the large number of spikes occasionally recorded from the muscles are explained as being longer volleys of pulses designed perhaps to enhance the strength of the downstroke. In any event it is clear that the number of pulses in the volley would not necessarily need to be very critical as a volley of from 5 - 9 pulses would in all likelihood produce equivalent responses from the dorsal longitudinal muscles.

Thus it is visualized that the flight centres of *Nudaurelia* are different ~~to~~ from those of *Theretra*, and the other insects with synchronous flight, in emitting *volleys* of pulses for each wing stroke, a situation which is probably associated with the exceptionally long duration and power of the wing beat stroke in the emperor moths.

SUMMARY.

1. Insects with a high wing beat frequency have been extensively studied; in this investigation the characteristics of the flight motor in a saturniid moth, *Nudaurelia cytherea capensis* Stoll. which has an extremely low wing beat frequency have been investigated.

2. The anatomy and innervation of the dorsal longitudinal flight muscles of this moth have been described.

3. A critical discussion of Tiegs's (1955) account of the histological appearance of insect flight muscles, and their classification into histological categories, is included. It is concluded after an investigation into the histological appearance of the flight muscles of *Nudaurelia* that these muscles are of the "close packed" type but are atypical in containing fibrils of unusually large size.

4. Two aspects of the neuromuscular physiology of the flight motor in this insect have been considered.

5. Electrical recording from the dorsal longitudinal flight muscles during normal flight have revealed that *Nudaurelia* is unique among insects studied to date, in having several action potentials associated with each wing stroke. It is suggested that this situation is related to the need for a powerful and prolonged contraction throughout the "power" or downstroke of the wing beat.

(i) It is suggested that the several muscle action potentials recorded from the dorsal longitudinal muscles during one stroke might be a reflection of a sequential contraction of the five dorsal longitudinal muscle blocks. Several lines of evidence are presented which support this idea.

(ii) The implications of this suggestion are considered in relation to those insects with a high stroke frequency in which only a single action potential is recorded from the flight muscles for each wing stroke. An example of this is given by the sphingid moth _Theretra capensis_ which has been briefly studied.

(iii) It was concluded that the idea of a sequential contraction of muscle blocks of _Nudaurelia_ is unacceptable and an alternative and more likely interpretation proposed. The latter visualizes the central nervous system emitting volleys of impulses which maintain a tetanus for the duration of one wing stroke.

6. The phenomenon of warm-up in _Nudaurelia_ is studied.

(i) The warm-up period is described and recordings made from the dorsal longitudinal muscles during this period are discussed.

(ii) Experiments are described which suggest the presence of a "warm-up centre" located in the prothoracic ganglion of the central nervous system. The probable innervation pathways to the flight muscles from this warm-up centre are described.

(iii) The results of these experiments also carry implications about the location of two postulated "flight centres" in the central nervous system. These implications are discussed.

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