

A COMPARATIVE STUDY OF THE STRUCTURE AND
BIOCHEMICAL ACTIVITY OF FLIGHT MUSCLES FROM SEVERAL
INSECT SPECIES

Dissertation submitted to the Graduate Faculty of the
Virginia Polytechnic Institute
in candidacy for the degree of
Doctor of Philosophy
in
Entomology
by
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February, 1965
Blacksburg, Virginia

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TABLE OF CONTENTS

	Page
INTRODUCTION.....	8
LITERATURE REVIEW.....	13
I. RESPIRATORY METABOLISM OF INSECT FLIGHT MUSCLES.....	13
A. Respiratory activity.....	13
B. Oxidative phosphorylation.....	17
C. The control of respiratory metabolism	19
1. Respiratory control by the phosph- ate acceptor.....	19
2. Respiratory control by the substrate level.....	21
3. Other factors affecting respirati- on.....	22
II. HISTOLOGY OF INSECT FLIGHT MUSCLES.....	25
MATERIALS AND METHODS.....	30
I. BIOLOGICAL AND CHEMICAL MATERIALS.....	30
II. BIOCHEMICAL METHODS.....	31
A. Preparation of mitochondrial suspens- ions.....	31
B. Incubation medium.....	32
C. Measurement of respiration and oxidative phosphorylation.....	33
D. Determination of mitochondrial prote- in.....	34
E. Permeability Studies.....	34

III. HISTOLOGICAL METHODS.....	34
A. Preparation of wet mounts.....	35
B. Preparation of permanent mounts.....	36
RESULTS.....	37
I. BIOCHEMICAL RESULTS.....	37
A. Respiratory Activity.....	37
B. Oxidative Phosphorylation.....	40
C. Respiratory Control.....	40
D. Permeability Studies.....	49
II. HISTOLOGICAL RESULTS.....	62
DISCUSSION.....	91
SUMMARY AND CONCLUSIONS.....	103
ACKNOWLEDGEMENTS.....	108
BIBLIOGRAPHY.....	110
VITA.....	116

LIST OF TABLES

Table		Page
1.	Average respiratory rates of mitochondria isolated from several insect species.....	38
2.	Rates of respiration and oxidative phosphorylation with mitochondria isolated from <u>Periplaneta americana</u>	41
3.	Rates of respiration and oxidative phosphorylation with mitochondria isolated from <u>Libellula luctuosa</u>	42
4.	Rates of respiration and oxidative phosphorylation with mitochondria isolated from <u>Protoparce sexta</u>	43
5.	Rates of respiration and oxidative phosphorylation with mitochondria isolated from <u>Corydalis cornutus</u>	44
6.	Rates of respiration and oxidative phosphorylation with mitochondria isolated from <u>Musca domestica</u>	45
7.	Rates of respiration and oxidative phosphorylation with mitochondria isolated from <u>Musca autumnalis</u>	46
8.	Rates of respiration and oxidative phosphorylation with mitochondria isolated from <u>Apis mellifera</u>	47
9.	Rates of respiration and oxidative phosphorylation with mitochondria isolated from <u>Popillia japonica</u>	48
10.	Effect of sonication treatments on the respiration of isolated sarcosomes from <u>Periplaneta americana</u>	50
11.	Effect of sonication treatments on the respiration of isolated sarcosomes from <u>Musca autumnalis</u>	51
12.	Effect of sonication treatments on the respiratory activity of isolated mitochondria from <u>Periplaneta americana</u> and <u>Musca autumnalis</u> , when prepared and incubated in a medium without serum albumin.....	54

13.	Effect of sonication treatments on the respiratory activity of isolated mitochondria of <u>Periplaneta americana</u> and <u>Musca autumnalis</u> , which were isolated and prepared in a medium consisting of 0.154 M KCl - 0.001 M EDTA (pH=7.4).....	55
14.	Effect of freezing and thawing on the respiratory activity of mitochondria isolated from <u>Periplaneta americana</u> and <u>Musca autumnalis</u> flight muscles.....	57
15.	Effect of a preincubation treatment with 0.006 M deoxycholate on the respiratory activity of mitochondria isolated from <u>Periplaneta americana</u>	59
16.	Cofactor and ADP effect on the respiratory activity of mitochondria isolated from <u>Apis mellifera</u>	60
17.	Cofactor effect on the respiratory activity of mitochondria isolated from <u>Apis mellifera</u>	61
18.	Effect of diphosphopyridine nucleotide (DPN) on the respiratory activity of mitochondria isolated from <u>Periplaneta americana</u>	63
19.	Some histological characteristics of freshly isolated flight muscles of several insect species.....	64

LIST OF FIGURES

Figure	Page
1. Effect of ultrasonic treatments on the respiratory rates of sarcosomes isolated from flight muscles of <u>Periplaneta americana</u> and <u>Musca autumnalis</u> with succinate as substrate.....	52
2.&3. Longitudinal views of freshly isolated fibers from the Basalar (Fig.2) and Tergo-pleural (Fig. 3) flight muscles of the male roach, <u>Periplaneta americana</u> . X4365.....	67
4.&5. Longitudinal views of freshly isolated fibers from the Dorsolongitudinal (Fig.4) and Tergo-sternal (Fig. 5) flight muscles of the dragonfly, <u>Libellula luctuosa</u> . X5529.....	69
6.&7. Longitudinal views of freshly isolated fibers from the Dorsolongitudinal (Fig. 6) and Tergosternal (Fig. 7) flight muscles of the moth, <u>Protoparce sexta</u> . Fig. 6, X7760; Fig. 7, X3880.....	71
8.&9. Longitudinal views of freshly isolated fibers from the Dorsolongitudinal (Fig. 8) and Tergosternal (Fig.9) flight muscles of the female dobsonfly, <u>Corydalis cornutus</u> . Fig. 8, X7333; Fig. 9, X5500....	73
10.&11. Longitudinal views of freshly isolated fibers from the Dorsolongitudinal (Fig. 10) and Tergosternal (Fig. 11) flight muscles of the housefly, <u>Musca domestica</u> X7566.....	75
12.&13. Longitudinal views of freshly isolated fibers from the Dorsolongitudinal (Fig.12) and Tergosternal (Fig.13) flight muscles of the face fly, <u>Musca autumnalis</u> . X5384...	77
14.&15. Longitudinal views of freshly isolated fibers from the Dorsolongitudinal (Fig. 14) and Tergosternal (Fig.15) flight muscles of the bee, <u>Apis mellifera</u> . Fig. 14, X7566 ; Fig. 15, X3783.	

- 16.&17. Longitudinal views of freshly isolated fibers from the Dorsolongitudinal (Fig.16) and Tergosternal (Fig.17) flight muscles of the japanese beetle, Popillia japonica X7178..... 81
- 18.&19. Longitudinal views of freshly isolated fibers from the Basalar (Fig.18) and Tergo-pleural (Fig.19) flight muscles of the female roach, Periplaneta americana. Fig. 18, X7272; Fig. 19, X3686..... 83
- 20.&21. Transverse sections of several groups of muscles (Fig. 20) and a close examination of a fiber (Fig.21) from the complex of the Tergo-pleural flight muscles of the male cockroach, Periplaneta americana. Fig. 20, X3589; Fig. 21, 7178..... 85
- 22.&23. Drastically (flat fibril in Fig. 22, arrow) and mildly (thick fibrils, grouped in Fig. 22 and isolated in Fig. 23) isolated fibrils from fibers of the flight muscles of the male cockroach, Periplaneta americana. Fig. 22, X3010; Fig. 23, X3395..... 87
24. Transverse section of a fiber from the Dorsoventral flight muscles of the dragonfly, Aeshna sp...... 89

INTRODUCTION

For many years insect flight has attracted the attention of workers in several scientific disciplines. Numerous investigations have been conducted in an effort to explain how this important phenomenon is accomplished. One of the things that has been learned is that not all insects have the same type of wing musculature. In this class, subcellular differences have been found among taxa as well as within the muscle machinery of a single insect (Tiegs, 1955; Boettiger, 1952, 1958, 1960; Howell, 1963). These different physical characteristics, which are thought to have appeared during the evolution of insects, are seemingly of a quantitative nature only, indicating that all types were probably derived from the same primitive muscle (Tubular) (Boettiger, 1960; Tiegs, 1955; Pringle, 1957).

Although a complete histological classification of the flight muscles in insects is still not available, a composite taxonomic arrangement of these muscles can be presented as follows:

1. Lamellar muscles
 - (a) Tubular (Odonata)
 - (b) Non-tubular (Blattidae)
2. Microfibrillar (close-packed) muscles (Lepidoptera, Neuroptera)
3. Fibrillar muscles (Diptera, Hymenoptera, Coleoptera)

Some intermediate types have been found between these three main groups (Tiegs, 1955; Pipa, 1955; Pringle, 1957; Howell, 1963).

From a physiological point of view, flight muscles can be separated in two main classes: 1. Those in which there is a "synchronous" (1:1) relationship between contraction response and motor nerve impulses. Included here are the "lamellar", and "close-packed" muscle types. 2. Those in which the rate of contraction is "asynchronous" with the nerve impulses (ratio greater than 1). To this group belong insects with the fibrillar type muscle (Roeder, 1951; Boettiger, 1960). The fibrillar type muscle can be further subdivided into at least two other physiological types, depending on their electrical behaviour (Boettiger, 1960).

Even though the flight muscle fibers were recognized as the cells of this tissue at the time of their discovery, it was not until very recently that the existence of mitochondria in the flight muscles was recognized (Watanabe and Williams, 1951). Since these sarcosomes were observed to be regularly arranged in rows between fibrils and parallel with respect to the muscle striations, their role in muscular contraction was immediately suspected. Hanson (1952) was able to demonstrate that isolated flight muscle fibrils from Dytiscus undergo contraction in the presence of ATP only when the sarcosomes adhere to the contracting fiber.

After these important findings a new concept in flight muscle research was created. For the first time it was possible to isolate these "unique" organelles from different insect sources, and study in vitro the role they play in the overall process of muscle contraction. As a result of these studies, it was soon learned that the mitochondria in flight muscles are the sites for the entire complex of respiratory enzymes which are concerned with the electron transport system and oxidative phosphorylation (Lewis and Slater, 1953; Sacktor, 1954).

In spite of these advances, conflicting reports have appeared in the literature concerning the respiratory and oxidative phosphorylation capacity of isolated insect mitochondria. In several cases, and even when the same species was used as the source of mitochondria, very wide differences were obtained. In the same vein, it should be pointed out that most of the work on flight muscle biochemistry has been accomplished with a relatively small number of insect species most of which have fibrillar type muscles for flight (Diptera).

In view of this situation, it appeared that a comparative study using a variety of insect species with different muscle types would be in order. In this way it would be possible to compare directly the results of biochemical analyses conducted under identical conditions but with mitochondria from different muscle types. Thus, the fear

that many of the past discrepancies are due to differences in general methodology could be eliminated. In addition, if corresponding histological examinations of the same muscles could be made in conjunction with the biochemical studies, new relationships might become evident. Accordingly, the present investigation was designed to fulfill these goals. Its objectives can be summarized as follows:

1. To determine the basic biochemical similarities and differences of mitochondria isolated from different types of flight muscles by comparing their respiratory and oxidative phosphorylation capacity under standard experimental conditions.

2. To make a comparative histological study of the known main types of flight muscle fibers to ascertain the typical arrangement, type and size of their subcellular components. Among these subcellular organelles are fibrils, mitochondria, and nuclei.

3. To correlate the biochemical activity of the mitochondria with the structural type which characterizes their neighboring fibrils in order to determine any metabolic implications that these specific functional associations might have in vivo.

Two corollary objectives were developed as the work progressed. These may be stated as follows:

1. To investigate the fibrillar arrangement of the flight muscle fibers in the cockroach, Periplaneta americana, in order to clarify any structural differences between these and the "tubular" type fibers present in the flight muscles of Odonata. These observations were accomplished by means of microhistological techniques.

2. To determine normal and induced differences in the permeability behaviour of isolated mitochondria under varying environmental conditions.

LITERATURE REVIEW

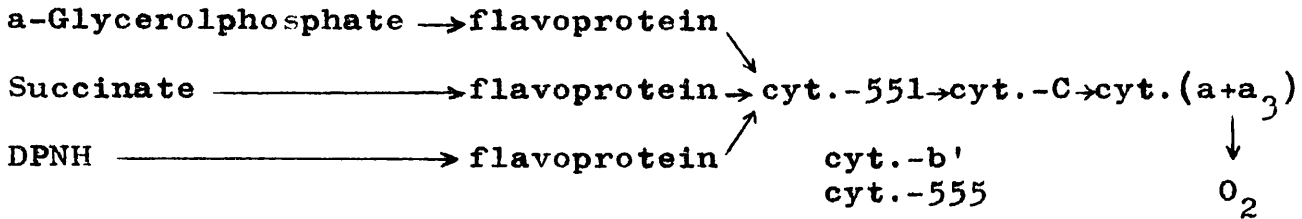
I. Respiratory Metabolism of Insect Flight Muscles

A. Respiratory Activity

Flight in insects is characterized by very rapid rates of energy transformation and oxygen consumption which take place in the flight muscles during muscular contraction. With the classical work of Keilin (1925), concerning the oxidation and reduction of cytochromes by the flight muscles of insects, the essential role that these pigments have in respiration was shown for the first time. This important finding was followed by the equally outstanding observation by Watanabe and Williams (1951) that the cytochromes are located in high titers in the sarcosomes of the flight muscles in Phormia regina. Once these investigators proved that the sarcosomes correspond to the mitochondria of other tissues, a series of detailed studies on the properties of the respiratory enzymes in these organelles followed (Levenbook and Williams, 1956; Sacktor, 1952; and many others).

Chance and Sacktor (1958), in a detailed spectroscopic study with isolated mitochondria from Musca domestica, were able to show that the respiratory chain in this fly is very similar to its counterpart in rat muscle mitochondria. Similar observations have been reported by Stegwee and Kammen-

Wertheim (1962) for isolated mitochondria from Leptinotarsa decemlineata. Based on these and many other studies (Chance, 1952; etc.), Estabrook and Sacktor (1958) postulated a scheme to describe the respiratory chain in insect mitochondria as follows:



Some pigments were indicated by their absorption wave length, and others (Cyt. b' and 555) were not located in the chain because their function is unknown. Another component of this chain is the recently discovered Coenzyme Q (Ubiquinone), which is widely distributed among plants and animals. Its exact site and function within the system is unknown (Lester and Crane, 1959).

When the number of sarcosomes per insect thorax (Levenbook and Williams, 1956) and the protein content in mitochondria (Watanabe and Williams, 1951) were estimated, it became possible to measure the respiratory rate in insects in a more meaningful way. This respiratory rate was called QO_2 , and it is defined as the amount of Oxygen consumed (ul.) per weight of mitochondrial protein (mg.) in a given time (hr.). When QO_2 's were estimated for the living insect, it was realized that they are much higher than those obtained for isolated mitochondria from flight muscles. QO_2 values sometimes higher than 500 were observed for Musca domestica in vivo (Chance and Sacktor, 1958).

Since these observations were made numerous studies on the oxidizing potential of isolated mitochondria have been carried out. When isolated sarcosomes from Musca were incubated in the presence of several substrates, it was shown that the highest QO_2 was obtained with α -glycerol phosphate. The activity of this substrate was 10 times greater than that of succinate, and 50 to 100 times greater than that of any other Krebs cycle intermediate (Chance and Sacktor, 1958; Sacktor, 1961). Although similar results in the oxidation of these substrates have also been reported with Musca by different investigators, the difference in activities were not as pronounced (Sacktor and Cochran, 1958; Estabrook and Sacktor, 1958; Gregg et al. 1960; Van den Bergh and Slater, 1962; Sacktor and Packer, 1961; Birt, 1961; Stegwee and Kammen-Wertheim, 1962). In agreement with these latter reports, comparable results have been shown with isolated mitochondria from Locusta (Zebe et al., 1959), Phormia (Sacktor, 1961), Leptinotarsa, Periplaneta* and Locusta (Stegwee and Kammen-Wertheim, 1962), Lucilia (Birt, 1961), and Periplaneta (Cochran and King, 1960; Cochran, 1961, 1962).

In light of these findings it was stated that α -glycerol phosphate is the physiological substrate for the activated

*In this case, the QO_2 for α -glycerol phosphate, succinate, α -ketoglutarate and glutamate; were 40, 38, 33 and 32, respectively

mitochondria during flight, and that the poor activity of the Krebs cycle intermediates suggested their secondary importance for this purpose (Chance and Sacktor, 1958; Sacktor, 1959, 1961, 1964). In opposition to this view, however, there is some information in the literature which indicates that some Krebs cycle intermediates are used as important energy sources for flight. Hoskins et al. (1956), using isolated mitochondria from Apis mellifera, demonstrated a complete oxidation of most of the Krebs cycle members to CO_2 and H_2O under special conditions. Van den Bergh and Slater (1961), working with Musca, stated that the high respiratory rates they obtained with α -glycerol phosphate ($\text{QO}_2=673$) and pyruvate+malate ($\text{QO}_2=245$) could be compared with the QO_2 calculated for this particular fly in vivo. In support to their suggestion they pointed out that a system oxidizing mainly α -glycerol phosphate leads to an accumulation or excretion of pyruvate, and this has never been observed in insects. In addition, these two substrates were the only sarcoplasmic substrates shown by these authors to penetrate rapidly into isolated intact sarcosomes. High pyruvate+malate oxidation rates ($\text{QO}_2=114$) with Musca sarcosomes have also been reported by Gregg et al. (1960).

Bucher, et al. (1959), contrary to the view of Chance and Sacktor (1958), Sacktor (1959, 1961, 1964), and Van den Bergh and Slater (1962), indicated that even when the ability of Locusta sarcosomes to oxidize α -glycerol phosphate is very high

as compared with other substrates, it was not enough to account for the total respiratory rate during flight. They reported that the activity of α -glycerol phosphate oxidase in Locusta sarcosomes is only 20% of the turnover number for cytochrome c during flight, and that pyruvate+malate produces a turnover number of only 10% of the flight rate. Similar results have been reported by Zebe, et al. (1959).

Relatively high respiratory rates have been reported for several Krebs cycle intermediates with isolated sarcosomes from Periplaneta (Cochran, 1963). The highest QO_2 (297), however, resulted from the oxidation of α -glycerol phosphate. An interesting finding in this study was the fact that the second highest QO_2 (132) was obtained with glutamate, one of the poorest substrates used by other workers (Stegwee and Kammen-Wertheim, 1962, with Leptinotarsa ($QO_2=32$); Birt, 1961, with Musca and Lucilia ($QO_2=10$); Van den Bergh and Slater, 1962, with Musca ($QO_2=20$)).

B. Oxidative Phosphorylation

Oxidative phosphorylation is an essential process which is common to all aerobic cells. This vital mechanism provides almost all of the ATP (Adenosine triphosphate) synthesized from ADP (Adenosine diphosphate) and Pi (Inorganic phosphate). This ATP generation takes place in the mitochondria by a series

of coupled reactions involving the electron transport system and at the expense of energy liberated in the biological oxidation of foodstuffs.

Oxidative phosphorylation in insects was first demonstrated by Lewis and Slater (1953) with isolated mitochondria from the flight muscles of Calliphora. Later, and working independently, Sacktor (1954) was able to demonstrate the existence of this process in isolated sarcosomes from Musca domestica. Subsequently, many other investigators have confirmed these findings with mitochondria isolated from the flight muscles of different insect sources. Important contributions to this field have been made by: Gonda et al. (1957) with Aedes, Chance and Sacktor (1958) with Musca, Ito and Horie (1959) with Bombyx, Wojtczak and Wojtczak (1959) with Galleria, Klingenberg and Bucher (1959) with Locusta, Cochran and King (1960) with Periplaneta, Gregg, et al. (1960) with Musca, Sacktor (1961) with Phormia, Birt (1961) with Lucilia and Musca, Stegwee and Kammen-Wertheim (1962) with Leptinotarsa, Locusta, Musca, and Periplaneta, Sacktor and Packer (1961) with Musca, Van den Bergh and Slater (1962) with Musca, and Cochran (1961, 1963) with Periplaneta.

The measure of oxidative phosphorylation efficiency in sarcosomes oxidizing different substrates is expressed as P:O ratios (number of umoles of phosphate esterified per uatom of oxygen consumed). From very extensive experimental work, it has been possible to determine approximately where and how

many sites occur in the respiratory chain, and which ones are involved in the synthesis of ATP when different substrates are being oxidized.

In mammalian systems, during the passage of an electron pair from an oxidized DPN-linked substrate to molecular Oxygen (terminal hydrogen acceptor) via the electron transport chain, 3 molecules of ATP are synthesized (4 with α -ketoglutarate). In the oxidation of non-DPN-linked substrates like α -glycerol phosphate and succinate, only 2 molecules of ATP are produced (Lehninger et al., 1958).

In insects P:O values from 2 to 3 have been reported for some DPN-linked substrates like glutamate, pyruvate and α -ketoglutarate. For succinate and α -glycerol phosphate (non-DPN-linked substrates), P:O ratios approaching 2 have been observed (Sacktor and Cochran, 1958; Cochran and King, 1960, Cochran, 1961, 1963; and several others).

C. The Control of Respiratory Metabolism

Changes in the respiratory rates between rest and flying conditions have been observed to be as high as 100-fold (Chadwick, 1953). How these dramatic changes are regulated is not yet well understood. In this section, therefore, a very brief review of some of the factors known or thought to be involved in the control mechanism will be discussed.

1. Respiratory control by the phosphate acceptor

It has been demonstrated in experiments with isolated mitochondria from different insect sources that the rate of oxidation of most Krebs cycle intermediates and pyruvate is under the definite control of phosphate acceptor (ADP) (Gonda et al., 1957; Klingenberg and Bucher, 1959; Gregg et al., 1960; Stegwee et al., 1962; Birt, 1961; Van den Bergh and Slater, 1962; and Cochran, 1963). The exception to this rule has been the oxidation of succinate where maximal oxidations with isolated mitochondria from Musca did not require the addition of ADP (Birt, 1961). In other reports succinate oxidation was even greater in the absence of this nucleotide (Cochran, 1963). Similar results have been reported in one instance for α -ketoglutarate (Sacktor and Cochran, 1958).

On the other hand, the effect of the phosphate acceptor on the oxidation of α -glycerol phosphate is very much in debate. In some cases this oxidation has been referred to as "loosely coupled" (Cochran and King*, 1960), or completely independent of the phosphate acceptor (Sacktor and Cochran*, 1958; Chance and Sacktor**, 1958; Cochran*, 1963). In other reports the oxidation of this substrate has been stimulated to various degrees by the presence of ADP in the reaction media

*Oxygen uptake was measured with a Warburg respirometer.

**Oxygen consumption was determined polarographically using the Oxygen electrode technique (Chance, 1954).

(Gonda et al.*, 1957; Bucher et al.*, 1959; Gregg et al.*, 1960; Van den Bergh and Slater*, 1962; Sacktor and Packer**, 1961; Birt*, 1961; and Stegwee et al.*, 1962). According to Chance (1959) the loss of respiratory control in insect sarcosomes is not necessarily followed by a decrease of their oxidative phosphorylation efficiency. In this case fairly high P:O ratios can be obtained with no measurable change of respiration in the presence or absence of a phosphate acceptor. Therefore, he concluded that the two phenomena are readily dissociable in intact mitochondria.

2. Respiratory control by the substrate level.

It has been stated that α -glycerol phosphate is the only substrate capable of sustaining the metabolic activities in actual flight (Chance and Sacktor, 1958; Sacktor, 1961). A strong support for this idea was provided by Winteringham (1958, 1959) when he found that moments after the initiation of flight the concentration of this substrate in the flight muscles decreased dramatically from a normally high level of 23% (of the total soluble phosphorous) to half this value. Similar results were obtained with Locusta (Bucher and Klingenberg, 1958). Therefore, and in the light of these results,

*Oxygen uptake was measured with a Warburg respirometer.

**Oxygen consumption was determined polarographically using the "Oxygen electrode technique" (Chance, 1954).

it was proposed that respiratory control in flight muscles of Musca was due to the regulation of substrate concentration or α -glycerol phosphate oxidase activity (Chance and Sacktor, 1958; Sacktor, 1958). Evidence for the control of respiration by regulation of this enzyme activity was given by Estabrook and Sacktor (1958) when they found that Ethylene diaminetetraacetate (EDTA - versene) blocks the oxidation of α -glycerol phosphate. This inhibition can be reversed by Mg^{++} or by additional substrate.

More recently in a detailed work with Phormia, Sacktor (1964) was able to demonstrate that an accumulation of pyruvate and the Krebs cycle intermediates did occur at the beginning of flight. Therefore, these results indicated that when flight begins the oxygen consumption is due mostly to the α -glycerol phosphate oxidation. Shortly thereafter the Krebs cycle members must be important in the continuation of flight.

3. Other factors affecting respiration

The great stimulatory effect of serum albumin on oxidative phosphorylation with isolated insect sarcosomes has been reported on several occasions (Sacktor, 1954; Wojtczak and Wojtczak, 1960). In some cases, however, it has been shown that oxidative phosphorylation can take place with or without this protein (Gregg et al., 1960; Cochran, 1961), and that a differential effect is possible depending on the substrate being oxidized (Van den Bergh and Slater, 1962; Cochran, 1963). The protective function of serum albumin is due to its ability to bind and

remove endogenous uncoupling agents (Wojtczak and Wojtczak, 1959) similar to some fatty uncouplers ("U" Factor) which are bound by this protein in mammalian liver mitochondria (Wojtczak and Lehninger, 1961).

Sacktor (1954) demonstrated that physical alterations on isolated sarcosomes, induced by the molarity of the isolation media, are closely associated with changes in oxidation, phosphorylation, and ATPase activity. According to this author, maximum coupling ability was obtained with 0.25M sucrose. When a solution of 0.25M sucrose+0.005M EDTA was used as the isolation media for Musca sarcosomes, Gregg et al. (1960) reported lower QO_2 values, lower acceptor ratios, and sometimes no indication of respiratory control for all substrates except pyruvate. Similar results were reported for Musca by Sacktor (1958) and Van den Bergh and Slater (1962). On the other hand, Stegwee and Kammen-Wertheim (1962), using a solution of 0.32M sucrose + 0.01M EDTA (pH=7.4), reported comparably good results with Leptinotarsa, Musca, Locusta, and Periplaneta sarcosomes.

Van den Bergh and Slater (1962) indicated that the highest QO_2 for α -glycerol phosphate and pyruvate were obtained when a solution consisting of 0.154 M KCl + 1 mM EDTA (pH=7.4) was used as the isolation medium for Musca sarcosomes. However, Cochran (1963) pointed out that the isolation of Periplaneta mitochondria in this same medium was completely inefficient in all preparations.

It has been observed that mitochondrial protein concentration, resulting from animals of different age, plays a decisive role in the outcome of respiration experiments and the response to ADP concentration (Lewis and Slater, 1954; Birt, 1961; and Cochran, 1963). In this respect Van den Bergh and Slater (1962) reported that the respiratory rate, P:O ratios, and respiratory control indexes obtained with sarcosomes from flies less than one day old were comparable to those obtained with flies older than 9 days.

The fact that isolated fly flight muscle mitochondria are not readily permeable to some Krebs cycle intermediates has been recognized for some time (Lewis and Slater, 1954). Recently, Van den Bergh and Slater (1962) were able to demonstrate that a permeability barrier exists in Musca mitochondria by treating isolated sarcosomes in a sonic disintegrator. As a result respiratory rate was increased with some Krebs cycle intermediates under special conditions.

In studies with sarcosomes isolated from Musca it is reported that maximal respiratory rates were obtained when a cofactor mixture (DPN⁺, TPP, TPN⁺, CoA, and Cytochrome c) was added to the normal reaction medium (Gregg et al., 1960). Sacktor (1954) indicated that the addition of cytochrome c had no effect on Oxygen consumption or phosphate uptake in mitochondria isolated from Musca. On the contrary, Stegwee et al. (1961) showed that the presence of this pigment (10^{-5} M) almost doubled the respiration rate when succinate was the substrate.

II. Histology of Insect Flight Muscles

A satisfactory classification of the flight muscles from the histological, physiological, and evolutionary points of view is still not available (Howell, 1963). However, some attempts at such a classification have been made.

Pringle (1957) recognized three types of histological evolution in the flight muscles of insects as follows:

1. Tubular muscles Leg and "normal" trunk muscles of some Orthoptera (Blattidae) and Odonata flight muscles.
2. Close-packed muscles Present in higher Orthoptera and Lepidoptera.
3. Fibrillar muscles Characteristic of Diptera, Coleoptera, and Hymenoptera.

A classification of the indirect flight muscles of insects based on the fibrillar arrangement of the muscle fibers was given by Pipa (1955) in the following manner:

1. Lamellar muscle Fibers characterized by the arrangement of the fibrils in flat sheets.
 - (a) Tubular Fibers with a central core of nuclei like the flight muscles in Odonata.
 - (b) Non-tubular Fibers with nuclei scattered throughout the body of the fiber (Blattidae and Mantidae).
2. Fibrillar muscles Fibers characterized by cylindrical or nearly cylindrical fibers.
 - (a) Microfibrillar Fibril size is about 1.5 μ in diameter.
 - (b) Fibrillar Fibril size is about 3 μ in diameter.

Among his results Pipa (1955) indicated that as a rule the fibril types of the families included within any particular order are similar, except in Orthoptera and Homoptera where either lamellar or microfibrillar are found in different species.

As can be seen from the above classifications, it is clear that insects can use different types of muscle other than the very specialized fibrillar type to sustain active flight. Although some structural characteristics of fibrillar muscle (large mitochondria, yellowish or brownish color, etc.) are frequently correlated with very active flight, none of these features appear to be specific and typical. Furthermore, some of these characteristics are lacking in notable cases, or they may be associated with other muscle types as well (Pipa, 1955). A remarkable example is that of Odonata where "giant sarcosomes" (up to 10 μ in length) are found associated with the primitive "tubular" muscles which are used by these insects to produce a very active flight (Smith, 1961).

In the evolution of the flight muscles changes in the physiological characteristics of these tissues were associated with changes in their histology (Tiegs, 1955). Thus, we can observe that in the case of lamellar (contrary to the findings of Edwards et al., 1954) and microfibrillar muscle types, fibril diameters are smaller than those of the thick fibrils of the fibrillar type. According to Snodgrass (1927), Pipa

(1955), and Tiegs (1955) the only histological feature of the fibrillar fibers which can be positively correlated with the production of a very rapid wing beat is that of its giant fibrils. Opposing this view, Jordan (1920) explained he could not find any correlation whatsoever between the ability to fly rapidly and the fibril diameter in Diptera, Hymenoptera and Coleoptera when contrasted with Orthoptera and Odonata. Instead, he suggested that the real correlation is between the duration of flight and fibril diameter. Giant fibrils, he indicated, may support a continuous and prolonged flight better than fibrils of a small diameter. Speculating on the significance of this correlation, Pipa (1955) suggested that probably the large size of fibrils permits a greater concentration of the contractile protein per unit area. In this respect Tiegs (1955) supported the idea that the flight muscles of higher insects are composed of fibers comparable to those of other types of muscle.

The "radial lamellar" muscles, with their typical strap-like fibrils, were first described by Aubert (1873), and since then they have been regarded as indivisible (Edwards et al., 1954; Pipa, 1955; Pringle, 1957). According to Keilich (1918), Marcus (1921), Jordan (1920), and Tiegs (1955) these "lamellar" fibers instead of having their fibrils arranged as flat sheets in a radial arrangement are, in some instances, actually present in rows or groups of small cylindrical or nearly cylindrical fibrils bound into a unit which gives the

appearance of a single flat structure. These composite fibrils Tieggs called "sarcostyles". In support of his views, Tieggs (1955) presented a large series of micrographs showing the existence of composite fibrils (sarcostyles) in the "lamellar" (Blattidae: Periplaneta americana) as well as in the fibrillar (Diptera: Asilidae) type muscles.

Based on the distribution of these sarcostyles (composite fibrils), Tieggs believed that in the evolution of higher or intermediate groups of insects (where the tendency was to develop a very high wing vibration with a minimum deformation of the thorax) the sarcostyles in the primitive "lamellar" muscle went through a remarkable thickening. As a result, the "coarse" fibrils of the fibrillar muscle (as first described by von Siebold, 1848) made their appearance to produce an apparently novel muscle.

Histological evidence for this "sarcostyle" evolution is found among the flight muscles of Homoptera and Orthoptera. In Cicadidae, nearly cylindrical fibrils are secondarily arranged in a complex lamellae. Cercopidae and Jassidae have flight muscles which combine characteristics from the close-packed and fibrillar types. This type of muscle has been called "pseudo-fibrillar." Blattella germanica and other Orthopteroids have their flight muscles mainly in the "tubular" condition, but some muscle fibers in these species exhibit a mixed arrangement of peripheral lamellae and a central core of small myofibrils (Tieggs, 1955).

Pipa (1955), after a careful examination of the Lamellar fibers in Periplaneta americana, concluded that the contractile material in this tissue is arranged in flat sheets instead of cylindrical fibrils. Elaborating on this particular point, Smith (1961) stated that if Tieg's suggestion of a composite fiber in the "radial lamellar" type muscle proved to be correct, this in no case could apply to Odonata or to Spider Leg muscles. He presented, in support to this statement, a series of light and electron micrographs showing the tubular arrangement of the dorsoventral muscles of Aeshna sp. (Odonata).

MATERIALS AND METHODS

I. Biological and Chemical Materials

The following is a list of the test animals involved in this investigation together with certain other relevant information. Adult insects were used in all cases.

Insect Species	Sex	Age(days)	Source
<u>Periplaneta americana</u>	males	>7	lab. cultures
<u>Libellula luctuosa</u>	mixed population	unknown	field collection
<u>Protoparce sexta</u>	mixed population	"	" "
<u>Corydalis cornutus</u>	female	"	" "
<u>Apis mellifera</u>	workers	"	apiary cultures
<u>Popillia japonica</u>	mixed population	"	field collection
<u>Musca domestica</u>	mixed population	4-7	lab. cultures
<u>Musca autumnalis</u>	mixed population	4-7	lab. cultures

A listing of the specialized chemical substances used in this work together with the source of supply is as follows: glutamic acid, malic acid, pyruvic acid (Sodium salt), a-ketoglutaric acid, succinic acid and pyridoxal phosphate were products from the California Corporation for Biochemical Research; NAD (DPN), TPN (Coenz. II) and CoA were purchased from The Pabst Laboratories; a-glycerol phosphate (Sodium salt) was supplied by the Eastern Chemical Corporation; crystalline bovine serum albumin was provided by the Pentex, Inc.;

hexokinase (Type III), deoxycholic acid (Sodium salt) and cytochrome c (Type III, from horse heart) were obtained from the Sigma Chemical Company; ethylene diamine tetraacetic acid (EDTA) came from Versenes, Inc.; and glutathione (reduced) from the Nutritional Biochemicals Corporation. The standard chemicals used were of reagent grade. All solutions were prepared with de-ionized distilled water.

II. Biochemical Methods

A. Preparation of mitochondrial suspension

Mitochondria from all species were obtained exclusively from isolated thoraces. The separation of heads and abdomens was carefully executed to make sure that no part of the gastro-intestinal tract of the animal remained attached to the thorax. As soon as the thoraces were separated they were placed in a beaker containing ice cold 0.25M sucrose in an ice bath. Once the desired number of thoraces was obtained, the rest of the preparation procedure was conducted in the cold according to the method described by Cochran (1961, 1963) An outline of this method is as follows:

1. Isolated thoraces were placed in a cold mortar where they were gently crushed with a pestle in the presence of small amounts of 0.25M sucrose.

2. The resultant homogenate was strained through a cheesecloth pad. The residue was further extracted with additional amounts of sucrose to obtain a better sarcosomal yield.

3. The mitochondrial suspension was centrifuged at 2,000 xG for approximately 1 minute and the precipitate discarded.

4. The supernatant fraction was centrifuged at 5,000 xG for 10 minutes and the supernatant discarded.

5. The residue (mitochondrial pellet) was resuspended in 0.25M sucrose containing 2.0% bovine serum albumin and centrifuged at 5,000 xG for 10 minutes.

6. The pellet was re-washed twice followed by centrifuging at 5,000 xG after each wash.

7. The last pellet (pure mitochondria) was resuspended in the necessary amount of 0.25M sucrose to be used in the experiment.

B. Incubation Medium

The basic incubation medium used in these experiments was standard for all test species. Its chemical composition was as follows: Mg Cl₂, 20 umoles; phosphate buffer (pH=7.4), 50 umoles; glucose, 50 umoles; hexokinase, 150 K.M. units; 5M KOH, 0.15 mls. (in center well); bovine serum albumin (BSA), 2%; substrate*, 60 umoles; adenosine diphosphate (ADP), 2 umoles; mitochondrial suspension (3.5 to 4.8 mgs of protein), 0.5 mls.; sucrose, 0.25M, the necessary amount to obtain a final volume of 2.5 mls. per flask.

*when the combination pyruvate+malate was used, 60 umoles of pyruvate+3 umoles of malate were added.

C. Measurement of Respiration and Oxidative Phosphorylation

Oxygen consumption was measured in a conventional Warburg constant volume respirometer. The temperature in the Warburg water bath was automatically maintained at 25°C, and the gas phase was air. After an equilibration period of 5 minutes (2 minutes for α -glycerol phosphate), the reaction was started by tipping the hexokinase-Glucose mixture from the side arm into the main compartment, and closing the system 5 minutes later (with α -glycerol phosphate the system was closed first and immediately thereafter the hexokinase-glucose solution was tipped in). An incubation period of 15 minutes was allowed for all substrates except α -glycerol phosphate for which this period was only 12 minutes. Manometer readings were made every 5 minutes (every 2 minutes for α -glycerol phosphate), and one thermobarometer was used in each Warburg run.

At the end of the experiment the reaction was stopped by adding to the reaction mixture 0.4 mls. of cdd 50% Trichloroacetic acid (TCA). The contents of each experimental flask were centrifuged to separate the protein. The disappearance of inorganic phosphate from an aliquot of the final supernatant fraction by the method of Fiske and SubbaRow (1925) was used to measure the amount of phosphorylation which occurred.

Oxidative phosphorylation was expressed as P:O ratios, and these were obtained by dividing the number of umoles of

inorganic phosphate esterified (amount of phosphorylation) by the number of atoms of Oxygen consumed by isolated sarcosomes incubated in the presence of a particular substrate.

QO_2 values were calculated as the microliters of Oxygen consumed per milligram of mitochondrial protein per hour. In this estimation only those respiratory rates which were linear with respect to reaction time were used.

D. Determination of Mitochondrial Protein

Protein determination in the sarcosomal fraction was accomplished by the colorimetric method of Lowry et al. (1951). In this estimation the standard protein was crystalline bovine serum albumin.

E. Permeability Studies

In experiments where the effect of sonic treatments on the permeability behaviour of isolated sarcosomes was tested, a Raytheon sonic oscillator (250 W. - supply 115 V.; 10KC- 60 cycles) was used. The output current employed in all tests was 1-1.5 amperes.

III. Histological Methods

To make a detailed histological study of the different cellular organelles in the fibers of flight muscles, it was

necessary to prepare these muscles for microscopic examination. This was done in slide preparations which were either temporary or permanent, depending on the observations to be made.

A. Preparation of Wet Mounts

The best isolation medium for the dissection work, was found to be 0.25M sucrose. Slide (wet) preparations made with this solution could be kept for observation at room temperature for periods of time over three hours without any detrimental effect on the morphology of the isolated fibers or their component organelles. These wet preparations were made as follows:

Once the desired muscle was dissected out of the thorax, representative bundles of fibers (muscle cells) were suspended in a small drop of 0.25M sucrose on a microscope glass slide. This muscle preparation was then covered by slowly dropping a glass cover on it and carefully pressing it just enough to spread evenly the drop of sucrose. A slide so prepared was then examined under a Bausch and Lomb compound light microscope. Representative fields were photographed with an EXA 35 mm. single lens reflex camera. The film used was Kodak Plus-X pan, and the light source was that provided with the microscope.

B. Preparation of permanent mounts

In order to determine with a better criterion the exact muscle type of some species, micro-histological techniques were employed to observe the cross-sections of these muscles in more detail. The preparation technique for this type of mount was as follows:

Once the desired muscles were dissected out of the thorax, they were fixed in Gilson's fixative for 1-1½ hours after which they were carefully washed in tap water several times to remove the fixing agents. The clean tissues were then subjected to an alcoholic dehydration in ascending concentrations of ethyl alcohol, and they were cleared later with Xylol (xylene) to remove the alcohol.

When this treatment was over, the tissues were infiltrated first with melted medium paraffin (Fisher's "Tissuemat", m.p. 52.5°C, kept in an oven), and then with melted hard paraffin (m.p. 56°C) in a vacuum chamber. Once the tissues were thoroughly permeated, they were properly oriented and imbedded with the same melted hard paraffin in a Stender dish. The paraffin blocks were allowed to harden. Sectioning of these blocks was then accomplished with a standard "Rotary microtome", and the paraffin sections (10 u thick) were affixed to glass slides previously albumenized (Mayer's albumen). After the slides were mounted they were stained using the Iron-haematoxylin-eosin technique. Examination of the slides was accomplished in a compound light microscope, and photographs were taken as described earlier.

RESULTS

I. Biochemical Results

A detailed account of the biochemical results is presented in Tables 1 through 18 and Figure 1. The respiratory rates employed in these calculations were those obtained during a 15 minute incubation period when respiration was linear with respect to incubation time. In all cases where α -glycerol phosphate was the substrate, a period of 8 to 10 minutes was used since the oxidation of this compound became non-linear after 10 minutes of incubation. When respiratory control by phosphate acceptor was investigated, adenosine diphosphate (ADP) was either added to or omitted from the standard reaction medium.

A. Respiratory Activity

In general, the results obtained on oxidation rates of freshly prepared sarcosomes from all insects studied were comparable to those obtained with insects by other workers and were applicable for mammalian systems as well (Sacktor and Cochran, 1958; Cochran and King, 1960; Gregg et al., 1960; Van den Bergh and Slater, 1962; Cochran, 1963; and others). The respiratory rates of isolated sarcosomes from all test species were commonly highest when α -glycerol phosphate was the substrate. The QO_2 values with this substrate ranged from 178-240 with sarcosomes from Periplaneta giving the highest value, while the lowest QO_2 was from Libellula sarcosomes (Table 1).

Table 1. Average respiratory rates of mitochondria isolated from several insect species.

Species	Succinic Acid	Glutamic Acid	Pyruvate +Malate	a-Ketoglutaric Acid	a-Glycerol Phosphate
<u>Periplaneta americana</u>	98.4±6.8*	131.2±5.7	122.4±6.1	111.6±4.1	240.0±5.7
<u>Libellula luctuosa</u>	130.0±6.3	44.2±7.3	31.3±4.5	26.0±5.6	178.0±8.3
<u>Protoparce sexta</u>	154.8±7.3	76.8±7.0	120.8±7.5	109.8±6.4	220.5±3.2
<u>Corydalis cornutus</u>	143.6±5.3	68.0±5.1	75.2±4.1	78.4±4.8	219.0±3.7
<u>Musca domestica</u>	136.1±3.6	33.6±4.8	58.6±3.9	36.8±4.1	202.5±7.3
<u>Musca autumnalis</u>	115.6±6.6	20.0±3.2	54.4±5.4	23.0±3.0	193.0±4.9
<u>Apis mellifera</u>	140.8±6.6	31.2±5.8	33.0±4.7	32.0±5.9	185.3±4.5
<u>Popillia japonica</u>	114.4±7.1	37.6±4.2	57.2±5.6	33.0±4.9	210.0±5.8

*All values reported here are expressed as QO_2 (ul. O_2 /mg. protein/hr.) and are the average of at least 7 experiments. The number after the \pm is standard deviation.

When sarcosomes from all species were incubated in the presence of glutamic acid, it was found that this substrate was oxidized at the lowest rate of all substrates tested except when Periplaneta sarcosomes were used. In this species glutamate had the second highest QO_2 (Table 1). This result confirmed previous observations by Cochran (1963) who also worked with Periplaneta.

The oxidation rates obtained for the combination of substrates pyruvate+malate, with mitochondrial preparations from all species, fluctuated widely. The highest QO_2 values were those obtained with sarcosomes from Periplaneta and Protoparce, while the lowest QO_2 were those from Libellula and Apis mitochondria. When the QO_2 's obtained for this combination were compared with those of other substrates oxidized by each species, it was found that respiratory rate was third or fourth highest in each preparation (Table 1).

The oxidation of the Krebs cycle intermediates succinate and a-ketoglutarate was different in some respects. As indicated in Table 1, with all mitochondrial preparations except those of Periplaneta the respiratory rates observed with succinate were the second highest and were all greater than 100. With Periplaneta sarcosomes the lowest QO_2 was obtained with succinate (Table 1).

When the metabolic substrate tested was a-ketoglutaric acid the respiratory rates fall roughly into two classes. High rates were observed with Periplaneta, Protoparce and

Corydalis, while all others showed low rates. This is an interesting dichotomy which seems to have some validity for all DPN-linked substrates tested (Table 1).

B. Oxidative Phosphorylation

Although the respiratory rates obtained with all species in the oxidation of α -glycerol phosphate were very high, they were coupled to a somewhat lower phosphorylation activity than might be expected. P:O values for this substrate varied from 0.9 (Musca domestica and Apis) to 1.3 (Periplaneta Protoparce and Libellula). In the case of succinate high P:O ratios could be obtained for Periplaneta (1.6) and Protoparce (1.5). The lowest P:O value for this substrate was 0.8 (Musca domestica and M. autumnalis). Normal P:O ratios (2.9-3.1) were common for pyruvate+malate, α -ketoglutarate, and glutamic acid (See Tables 2-9).

C. Respiratory Control

Respiratory control by phosphate acceptor (ADP) was not demonstrated for α -glycerol phosphate and succinate in any species tested, nor for pyruvate+malate in Apis since QO_2 values were essentially the same with or without this nucleotide. With all the DPN-linked substrates (glutamic acid, pyruvate+malate and α -ketoglutaric acid), however, a rather tight respiratory control was demonstrated in all

Table 2. Rates of respiration and oxidative phosphorylation with mitochondria isolated from Periplaneta americana.

Time (Mins.)	Succinate		Glutamate		Pyruvate		a-Ketoglutarate		a-glycerol phosphate	
	+ADP	-ADP	+ADP	-ADP	+ADP	-ADP	+ADP	-ADP	+ADP	-ADP
5 (4)*	2.3	2.8	2.7	0.4	3.1	0.5	2.6	0.3	4.3	4.1
10 (8)	5.6	6.1	7.3	0.7	7.2	0.8	6.3	0.7	11.6	10.9
15 (12)	9.0	9.5	12.0	1.0	11.2	1.2	10.2	1.1	13.8	13.8
QO ₂	98.4	103.6	131.2	10.8	122.4	12.6	111.6	11.2	240.0	226.0
P/O	1.6		3.0		3.1		3.0		1.3	

*The number in parenthesis refers to the reaction time for a-glycerol phosphate.

Table 3. Rates of respiration and oxidative phosphorylation with mitochondria isolated from Libellula luctuosa

Time (Mins.)	Succinate		Glutamate		Pyruvate + Malate		a-Ketoglutarate		a-Glycerol Phosphate	
	+ADP	-ADP	+ADP	-ADP	+ADP	-ADP	+ADP	-ADP	+ADP	-ADP
5 (4)*	3.5	3.7	1.3	0.3	0.7	0.4	0.5	0.3	2.6	2.5
10 (8)	6.7	6.8	2.3	0.8	1.6	0.8	1.2	0.8	7.3	7.0
15 (12)	10.0	10.0	3.4	1.5	2.4	1.3	2.0	1.3	9.1	9.0
QO ₂	130.0	130.0	44.2	19.0	31.3	16.5	26.0	16.2	178.0	170.3
P/O	1.2		3.0		3.0		3.0		1.3	

*The number in parenthesis refers to the reaction time for a-glycerol phosphate

Table 4. Rates of respiration and oxidative phosphorylation with mitochondria isolated from Protoperca sexta

Time (Mins.)	Succinate		Glutamate		Pyruvate		a-Ketoglutarate		a-Glycerol Phosphate	
	+ADP	-ADP	+ADP	-ADP	+ADP	-ADP	+ADP	-ADP	+ADP	-ADP
5 (4)*	4.8	4.4	2.2	0.1	3.4	0.4	2.8	0.2	3.8	3.5
10 (8)	9.4	8.5	4.6	0.3	7.2	0.7	6.4	0.5	10.7	10.5
15 (12)	14.1	12.2	7.0	0.5	11.0	1.2	10.0	0.8	13.0	12.7
QO ₂	154.8	134.0	76.8	4.8	120.8	13.2	109.8	8.8	220.5	210.0
P/O	1.5		3.0		3.1		3.0		1.3	

*The number in parenthesis refers to the reaction time for a-glycerol phosphate

Table 5. Rates of respiration and oxidative phosphorylation with mitochondria isolated from Corydalis cornutus

Time (Mins.)	Succinate		Glutamate		Pyruvate +Malate		a-Ketoglutarate		a-Glycerol Phosphate	
	+ADP	-ADP	+ADP	-ADP	+ADP	-ADP	+ADP	-ADP	+ADP	-ADP
5 (4)*	4.5	5.0	1.1	0.3	1.7	0.5	2.4	0.4	4.7	4.5
10 (8)	9.4	10.0	3.9	0.5	4.6	1.3	4.2	1.2	11.6	10.9
15 (12)	14.3	15.0	6.7	0.8	7.5	1.9	7.0	1.9	13.0	13.2
QO ₂	143.6	150.8	68.0	7.6	75.2	19.0	78.4	19.0	219.0	218.6
P/O	1.2		3.0		3.0		3.1		1.2	

*The number in parenthesis refers to the reaction time for a-glycerol phosphate

Table 6. Rates of respiration and oxidative phosphorylation with mitochondria isolated from Musca domestica

Time (Mins.)	Succinate +ADP	Succinate -ADP	Glutamate +ADP	Glutamate -ADP	Pyruvate +Malate +ADP	Pyruvate +Malate -ADP	a-Ketoglutarate +ADP	a-Ketoglutarate -ADP	β -Glycerol Phosphate +ADP	β -Glycerol Phosphate -ADP
5 (4)*	4.4	4.6	0.6	0.3	1.5	0.4	0.6	0.3	4.1	4.1
10(8)	8.7	9.2	1.9	0.6	3.6	0.8	2.1	0.6	10.0	10.6
15(12)	13.0	13.5	3.2	0.9	5.6	1.3	3.5	0.9	12.1	12.6
QO ₂	136.1	141.2	37.6	9.6	58.6	14.0	36.8	9.6	202.5	210.0
P/O	0.8		3.0		3.1		2.9		0.9	

*The number in parenthesis refers to the reaction time for a-glycerol phosphate

Table 7. Rates of respiration and oxidative phosphorylation with mitochondria isolated from Musca autumnalis

Time (Mins.)	Succinate		Glutamate		Pyruvate +Malate		a-Ketoglutarate		a-Glycerol Phosphate	
	+ADP	-ADP	+ADP	-ADP	+ADP	-ADP	+ADP	-ADP	+ADP	-ADP
5 (4)*	3.2	4.2	0.4	0.2	2.0	0.7	0.5	0.1	4.4	4.3
10 (8)	7.3	8.2	1.2	0.4	3.8	1.5	1.4	0.3	10.4	10.5
15 (12)	11.5	12.3	2.0	0.6	5.5	2.1	2.3	0.5	12.2	12.7
QO ₂	115.6	121.6	20.0	5.6	54.4	20.8	23.0	4.7	193.0	195.0
P/O	0.8		3.0		3.1		3.0		1.0	

*The number in parenthesis refers to the reaction time for a-glycerol phosphate

Table 8. Rates of respiration and oxidative phosphorylation with mitochondria isolated from Apis mellifera

Time (Mins.)	Succinate +ADP	Succinate -ADP	Glutamate +ADP	Glutamate -ADP	Pyruvate +ADP	Pyruvate -ADP	a-Ketoglutarate +ADP	a-Ketoglutarate -ADP	Glycerol Phosphate +ADP	Glycerol Phosphate -ADP
5 (4)*	5.0	5.1	0.9	0.2	0.9	0.9	0.8	0.4	3.6	3.9
10 (8)	10.1	10.2	2.1	0.4	2.1	1.9	2.0	0.7	10.5	10.9
15 (12)	15.0	15.0	3.3	0.5	3.5	3.1	3.4	0.9	12.2	13.0
QO ₂	140.8	140.8	31.2	4.8	33.0	30.0	32.0	8.4	185.3	195.0
P/O	0.9		2.9		3.0		3.0		0.9	

*The number in parenthesis refers to the reaction time for a-glycerol phosphate

Table 9. Rates of respiration and oxidative phosphorylation with mitochondria isolated from Popillia japonica

Time (Mins.)	Succinate		Glutamate		Pyruvate		a-Ketoglutarate		a-Glycerol Phosphate	
	+ADP	-ADP	+ADP	-ADP	+ADP	+ADP	+ADP	-ADP	+ADP	-ADP
5 (4)*	2.0	2.3	0.9	0.4	0.9	0.3	0.9	0.4	3.6	3.3
10 (8)	6.0	6.2	2.1	0.8	3.0	0.6	2.4	0.7	10.0	9.6
15 (12)	10.2	10.4	3.4	1.1	5.2	0.9	3.8	1.2	112.0	12.8
QO ₂	114.4	114.8	37.6	12.0	57.2	9.9	33.0	13.1	210.0	202.5
P/O	1.2		3.0		3.0		2.9		1.2	

*The number in parenthesis refers to the reaction time for a-glycerol phosphate

mitochondrial preparations (except with Apis as noted above). In these cases maximum respiratory rates were only possible in the presence of ADP.

D. Permeability Studies

The low oxidative ability of sarcosomes isolated from Musca autumnalis and Apis for Krebs cycle intermediates as well as glutamic acid was investigated further. In these experiments Periplaneta sarcosomes were used as the standard preparation since their oxidative capacity for all substrates was shown to be very high. The results of a series of tests designed to determine if these poor oxidations were due to permeability barriers in the active sarcosomes are summarized in Tables 10 through 15.

As shown in Tables 10, 11 and Figure 1, the effects of sonication treatments on isolated sarcosomes from Musca and Periplaneta were the same. In these experiments sonication was stimulatory on the oxidative potential of mitochondria only for succinate. This stimulatory effect on respiration was not observed when any other substrate was tested.

When the preparation and incubation of isolated sarcosomes from Periplaneta and Musca was accomplished in the absence of serum albumin, it was found that the respiratory activity of these sarcosomes was not modified since comparable QO_2 's were obtained with or without this protein.

Table 10. Effect of sonication treatments on the respiration of isolated sarcosomes from Periplaneta americana

Sonication Time	Succinic Acid	Glutamic Acid	Pyruvate +Malate	a-Keto-glutaric Acid	a-Glycerol Phosphate
0	92.8*	135.6	118.0	111.6	255.8
1 second	95.2	135.2	118.0	109.3	233.3
3 seconds	101.6	134.4	116.0	108.0	202.5
5 "	107.2	133.0	116.0	104.0	192.8
10 "	108.0	122.0	109.0	100.0	184.5
15 "	115.0	115.0	100.0	85.0	174.0
20 "	128.0	92.0	85.6	75.2	170.5
30 "	138.0	36.0	31.6	43.6	165.0
1 minute	146.0	18.6	27.2	24.0	164.3
1½ minutes	150.0	14.4	25.0	17.6	159.8
2 "	146.0	10.0	18.4	10.8	150.0
3 "	135.0	5.6	5.6	4.1	139.5
4 "	118.0	4.4	1.1	0.2	129.0
5 "	100.0	3.2	0.2	0.0	123.0
6 "	84.0	0.1	0.0	0.0	123.0
7 "	71.0	1.0	0.0	0.0	120.0
8 "	59.8	0.0	0.0	0.0	117.0
9 "	47.2	0.0	0.0	0.0	114.8
10 "	35.0	0.0	0.0	0.0	111.0

*All values are expressed as QO₂

Table 11. Effect of sonication treatments on the respiration of isolated sarcosomes from Musca autumnalis

Sonication Time	Succinic Acid	Glutamic Acid	Pyruvate Malate	a-Ketoglutaric Acid	a-Glycerol Phosphate	Malic Acid
0	94.0*	17.8	63.2	18.8	192.8	16.0
1 second	96.0	17.8	60.0	18.3	192.0	---
3 seconds	98.0	16.8	50.1	18.3	190.0	---
5 "	98.0	11.9	41.6	16.0	187.2	---
30 "	100.0	4.0	15.0	6.3	185.0	5.0
1 minute	106.0	4.0	6.0	5.7	176.0	3.0
2 minutes	115.0	3.0	4.5	4.8	172.5	2.0
3 "	124.0	2.1	3.9	4.0	168.0	2.0
4 "	118.0	1.0	2.0	3.2	153.8	1.0
5 "	108.0	1.0	1.0	2.0	113.7	0.0
6 "	100.0	0.0	1.0	0.9	96.0	0.0
7 "	90.0	0.0	0.0	0.0	94.5	0.0
8 "	78.0	0.0	0.0	0.0	90.0	0.0

*All values are expressed as QO_2

Explanation of Figure 1.

Effect of ultrasonic treatments on the respiratory rates of sarcosomes isolated from the flight muscles of Periplaneta americana and Musca autumnalis with succinate as substrate.

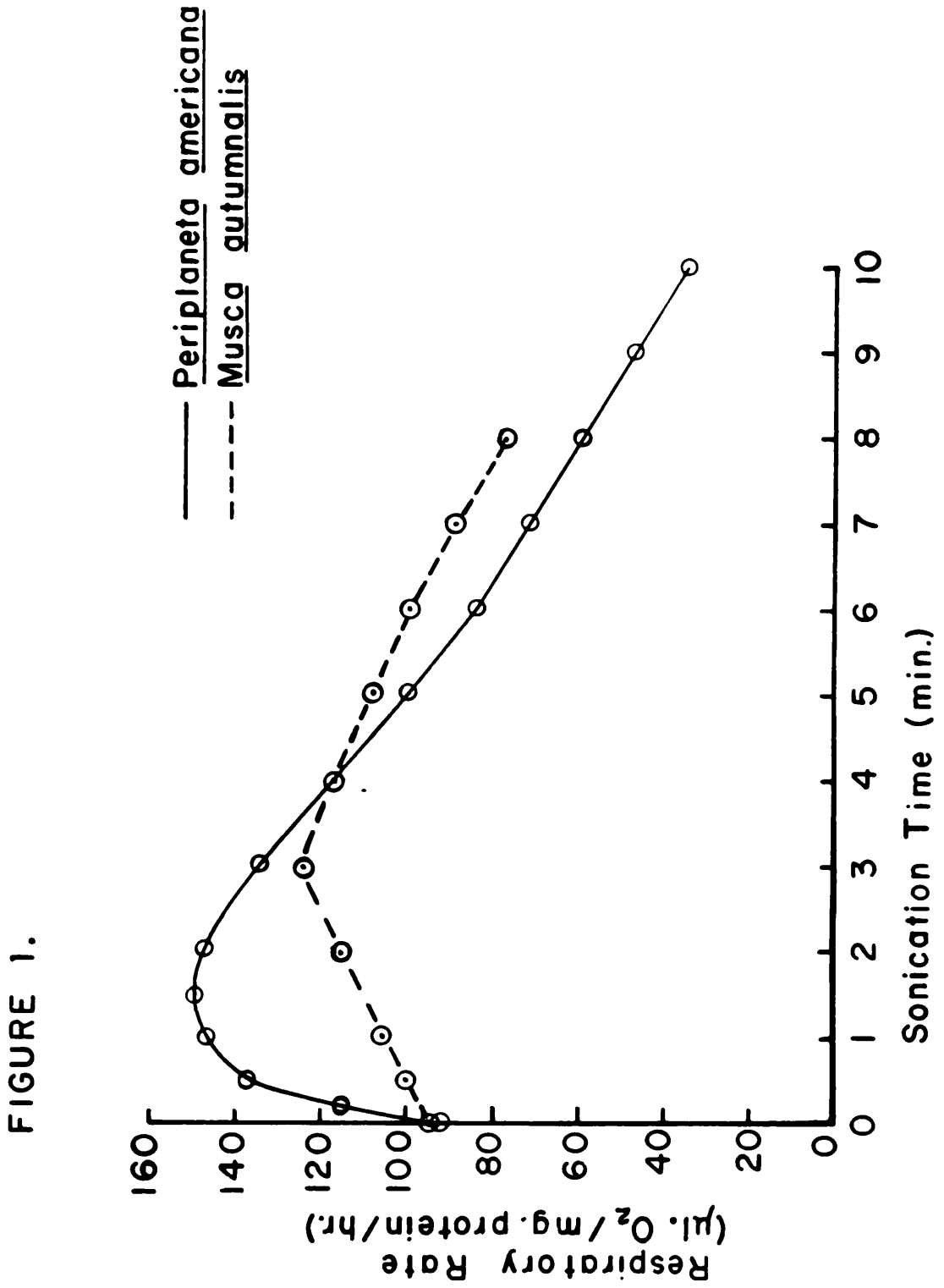


Table 12. Effect of sonication treatments on the respiratory activity (expressed as QO_2) of isolated mitochondria from Periplaneta americana and Musca autumnalis when prepared and incubated in a medium without serum albumin.

	Sonication Time (Min.)	Succinic Acid	Glutamic Acid	Pyruvate +Malate	a-Ketoglutaric Acid	a-Glycerol Phosphate
<u>Periplaneta</u>	0	108.0	120.0	120.0	104.8	235.0
<u>americana</u>	$\frac{1}{2}$	89.6	62.4	44.8	34.0	202.5
	$1\frac{1}{2}$	75.6	17.6	10.8	6.5	184.5
	3	59.2	3.2	0.0	0.0	159.8
	5	50.4	0.0	0.0	0.0	129.0
<u>Musca</u>	0	91.0	20.0	57.2	19.8	192.3
<u>autumnalis</u>	2	52.4	1.8	6.0	4.0	161.3
	4	49.6	0.0	2.0	0.0	135.8
	6	44.4	0.0	0.0	0.0	114.8

Table 13. Effect of sonication treatments on the respiratory activity (expressed as QO_2) of isolated mitochondria of Periplaneta americana and Musca autumnalis which were isolated and prepared in a medium consisting of 0.154 M KCl + 0.001 M EDTA (pH=7.4)

	Sonication Time (Min.)	Succinic Acid	Glutamic Acid	Pyruvate + Malate	a-Ketoglutaric Acid	Glycerol Phosphate
<u>Periplaneta</u>	0	52.4	75.6	144.0	66.8	202.0
<u>americana</u>	$\frac{1}{2}$	66.8	10.9	24.0	18.6	170.5
	$1\frac{1}{2}$	86.4	1.1	3.2	3.2	159.8
	3	60.0	0.0	0.0	1.1	119.0
	5	47.2	0.0	0.0	0.0	84.8
<u>Musca</u>	0	12.8	11.9	83.2	9.9	162.5
<u>autumnalis</u>	2	34.8	2.9	4.0	3.0	75.8
	4	26.8	1.0	1.0	2.0	48.8
	6	10.0	0.0	0.0	0.0	30.0

When these preparations from both species were further treated with a sonic disintegrator, the effect of sound was apparent in a sharp decrease in respiration in those sarcosomes not prepared with bovine serum albumin (Table 12). This was particularly evident when succinate was used as substrate (compare Tables 10 and 11 with 12).

The results of isolating and preparing sarcosomes in a medium consisting of a solution of 0.154 M KCl+0.001 M EDTA (sarcosomes were resuspended in 0.25 M in the final preparation) are shown in Table 13. In both species the oxidation of all the substrates was considerably decreased with the exception of pyruvate + malate where the rates were increased (compare 0 time in Table 12 and 13). If after this treatment sarcosomes from these species were subjected to sonication a rapid loss of their oxidative ability for all substrates always occurred, except for succinate where short term sonications actually stimulated its oxidation.

The results of freezing and thawing preparations containing sarcosomes from Periplaneta and Musca indicated that this treatment had a differential effect on the sarcosomes depending on the substrate being metabolized. As shown in Table 14, repeated or prolonged freezing stimulated the oxidative capacity of the sarcosomes for succinate and α -glycerol phosphate, but decreased considerably this capacity for the rest of the substrates.

Table 14. Effect of freezing and thawing on the respiratory activity (expressed as QO_2) of mitochondria isolated from Periplaneta americana and Musca autumnalis flight muscles.

Substrate	Untreated		Frozen and Thawed Once		Frozen and Thawed Twice		Frozen for 24 Hours	
	P.*	M.**	P.	M.	P.	M.	P.	M.
Succinic Acid	89.6	92.0	92.8	96.0	94.4	104.8	148.0	111.2
Glutamic Acid	124.0	16.0	121.6	15.2	119.6	15.2	60.0	8.4
Pyruvate+Malate	86.8	49.0	85.6	47.2	84.8	45.2	38.0	6.2
a-Ketoglutaric Acid	99.6	24.4	97.6	22.2	96.4	24.4	56.8	7.0
a-Glycerol Phosphate	195.8	150.0	202.5	160.0	203.0	167.5	225.0	175.0

*Periplaneta americana

**Musca autumnalis

The pre-incubation of Periplaneta sarcosomes with 0.006 M deoxycholate (in 0.25M sucrose) in the cold resulted in a decrease of the respiratory activity of these sarcosomes with all substrates tested (Table 15).

E. Cofactor Requirements

From the foregoing results it appeared that a rather consistent difference in rate of oxidation occurred between DPN-linked substrates and non-DPN-linked substrates for certain species. Therefore, the cofactor requirements of sarcosomes from Apis and Periplaneta were compared.

The cofactor requirements of isolated sarcosomes from Apis are shown in Table 16. These results indicate that the oxidation of glutamic acid was stimulated by the presence of this cofactor mixture at certain concentrations. If glutathione was added in the presence of these coenzymes, a small further increase in the oxidation of glutamate was observed. The respiratory rates for pyruvate+malate were consistently improved by most concentrations of the coenzyme mixture tested. Of interest was the fact that these stimulations were higher (in the case of pyruvate+malate) in the absence of ADP than in its presence. Further experiments (Table 17) showed that succinate oxidation was not stimulated

Table 15. Effect of a preincubation treatment with 0.006 M deoxycholate on the respiratory activity (expressed as QO_2) of mitochondria isolated from Periplaneta americana.

Substrate	Untreated	5 Minutes	15 Minutes	30 Minutes
Succinic Acid	101.0	66.8	50.4	35.6
Glutamic Acid	138.8	60.8	48.0	14.0
Pyruvate +Malate	86.0	30.4	21.6	18.8
α -Glycerol Phosphate	236.6	229.5	149.3	120.8

Table 16. Cofactor and ADP effect on the respiratory activity (expressed as QO_2) of mitochondria isolated from *Apis mellifera*.

Incubation Medium	Glutamic Acid +ADP	Glutamic Acid -ADP	Pyruvate +Malate +ADP	Pyruvate +Malate -ADP
Standard Medium	15.0±3.0*	2.0±2.0	16.0±1.0	20.0±2.8
Standard Medium+ 0.05 ml cofactor mixture**	7.0±1.0	0.0	25.0±2.2	30.0±4.4
Standard Medium+ 0.1 ml cofactor mixture	19.0±2.0	8.0±2.0	32.0±2.2	46.0±1.0
Standard Medium+ 0.2 ml cofactor mixture	14.0±3.0	4.0±1.0	39.0±3.6	50.0±5.0
Standard Medium+ 0.3 ml cofactor mixture	10.0±2.0	0.0	29.0±3.0	38.0±4.0
Standard Medium+ 0.5 ml cofactor mixture	5.0±1.0	0.0	11.0±2.0	24.0±3.5
Standard Medium+ 0.1 ml cofactor mixture	21.0±3.0			

* QO_2 ± the standard deviation.

**Cofactor mixture=(DPN, 0.15 uml.; TPN, 0.13um1.; CoA, 0.13um1.; cyt. c, 0.008um1; vit. B₆, 0.4um1/0.1ml)

Table 17. Cofactor effect on the respiratory activity (expressed as QO_2) of mitochondria isolated from Apis mellifera.

Incubation Media	Succinic Acid	Pyruvate+Malate
Standard Medium	127.8 \pm 6.0*	18.0 \pm 4.6
Standard Medium +cofactor mixture**	79.2 \pm 4.5	34.0 \pm 5.0
Standard Medium +Co A***	125.6 \pm 3.5	9.6 \pm 4.1
Standard Medium + DPN***	122.8 \pm 2.3	37.5 \pm 3.2
Standard Medium + TPN***	132.4 \pm 5.3	9.6 \pm 4.8
Standard Medium + Cyt. c***	131.6 \pm 5.4	18.0 \pm 4.0
Standard Medium +Pyridoxal phosphate***	50.8 \pm 6.4	9.6 \pm 1.7

* $QO_2 \pm$ the standard deviation.

**Cofactor mixture=(DPN, 0.30um1.; TPN, 0.26um1; CoA, 0.26um1.; cyt. c, 0.016um1.; Pyridoxal phosphate (vit B₆), 0.8um1).

***When these coenzymes were used singly, the concentration indicated above for each one was used.

by the cofactor mixture, and that the net stimulation observed with pyruvate+malate when this coenzyme concentrate was included was due only to the presence of DPN (NAD). When succinate was oxidized in the presence of pyridoxal phosphate, and pyruvate+malate in the presence of CoA, TPN, or pyridoxal phosphate, these coenzymes actually inhibited the oxidations.

When the stimulatory effect of DPN (NAD) on the respiratory activity of Periplaneta sarcosomes was investigated, it was found that the respiratory rates obtained with all substrates were the same with or without the addition of this coenzyme, except that in two instances the rates were improved in the absence of ADP (Table 18).

II. Histological Results

The results obtained in this part of the study are summarized in Table 19 and in Figures 2 through 24. All histological observations except those on the transverse sections of the male roach flight muscles were made exclusively on freshly isolated flight muscle fibers. In all cases, the fibrils and sarcosomes were readily visible in the slide preparations under a standard light microscope. For this reason the staining of the sarcosomes was not necessary. On the other hand, the preparation of cross-section slides of Periplaneta were critical and special staining techniques had to be employed to accomplish this type of fibrillar observations.

Table 18. Effect of Diphosphopyridine nucleotide (DPN) on the respiratory activity (expressed as QO_2) of mitochondria isolated from Periplaneta americana.

Substrates	Standard Medium			
	+ADP -DPN	-ADP -DPN	+ADP +DPN*	-ADP +DPN
Succinic Acid	104.2 \pm 5.6**	116.4 \pm 5.1	107.2 \pm 5.4	114.8 \pm 4.3
Glutamic Acid	99.6 \pm 4.7	17.2 \pm 3.6	100.0 \pm 6.0	38.0 \pm 4.7
Pyruvate +Malate	49.0 \pm 5.8	32.0 \pm 3.6	56.4 \pm 5.4	36.5 \pm 5.7
a-Ketoglutaric Acid	98.0 \pm 4.2	27.2 \pm 4.5	103.2 \pm 4.6	48.0 \pm 3.3

*When DPN was added to the incubation medium 0.30 umoles were used.

** QO_2 \pm the standard deviation.

Table 19. Some histological characteristics of freshly isolated flight muscles of several insect species.

Order	Species	Muscle Type	Fiber Diameter(u)	Fibril Diameter(u)	Approximate sarco- some size(u)
Orthoptera	<u>Periplaneta americana</u>	Pseudo-lamellar*	29-42	0.6-0.8	0.9-1.4
Odonata	<u>Libellula luctuosa</u>	Tubular-lamellar	22-32	0.6-0.8	2.4-3.5
Lepidoptera	<u>Protoparce sexta</u>	Microfibrillar**	70-150	0.7-1.3	1.5-2.5
Neuroptera	<u>Corydalus cornutus</u>	Microfibrillar	60-155	0.8-1.2	1.5-2.4
Diptera	<u>Musca domestica</u>	Fibrillar	----	2.0-3.0	1.7-4.6
Hymenoptera	<u>Apis mellifera</u>	Fibrillar	----	2.8-3.1	2.4-4.8
Coleoptera	<u>Popillia japonica</u>	Fibrillar	----	2.5-3.3	2.5-4.7

*The term pseudo-lamellar is proposed for this type of muscle.

**Close-packed of other authors.

Contrary to a report by Edwards et al. (1954) it was found in this work that the size of the fibrils of the flight muscle fibers of Periplaneta were notably smaller than those isolated from any of the fibrillar type fibers observed (see Table 19 and compare Figures 2 and 3 with 10 through 17).

In all muscle fibers observed there were numerous sarcosomes, and these were perfectly arranged in characteristic straight rows between the fibrils (Figures 2 through 19). Wide variations in the size of the mitochondria as well as in the coloration of the muscle were observed. The fibers were surrounded by a sarcolemma membrane in all species examined.

In disagreement with observations of Edwards et al. (1954) sarcosomes were found to be present in both male and female Periplaneta flight muscles. In the female roach, however, as shown in Figures 18 and 19, these sarcosomes are not as abundant as in the male. As can be observed by comparing Figures 2 and 3 with 18 and 19, the spaces between each row of sarcosomes is considerably greater in the females.

When the histological differences between the two types of lamellar fibers ("non tubular" or "pseudo-lamellar", and "tubular") was investigated, cross-sections of most of the red flight muscles of male Periplaneta were made as described earlier in the methods section. In these

transverse sections (see Figures 20 through 24) it is clear that all the red flight muscles of the male roach have the same type of "non-tubular" or more correctly "pseudo-lamellar" fiber. Although these two types of fiber show a similar polygonal arrangement characteristic of the "tubular" fibers (like in Odonata), they were slightly larger in the cockroach. In the "pseudo-lamellar" (non-tubular) fibers of Periplaneta the nuclei do not occupy a central core portion as in the "tubular" fiber, but instead they are distributed at random throughout the body of the fiber. In a large number of cases these nuclei appeared to be marginal and located just beneath the sarcolemma (see Figures 20, 21 and 24).

Contrary to the view of Pipa (1955), the lamellar components are resolvable not only with the aid of an electron microscope but also with the standard light microscope. As it is shown in Figures 20 through 24, it is quite evident that in the male roach muscle fibers the contractile organelles are of a small diameter and they are roughly cylindrical to oval in form. They are only secondarily arranged into irregular composite lamellae. The pseudo-lamellar arrangement of these small and independent fibrils is difficult to observe even in a good transversal section unless this preparation is properly stained.

Explanation of Figures 2 and 3

Longitudinal views of freshly isolated fibers
from the Basalar (Fig.2) and Tergo-pleural
(Fig.3) flight muscles of the male cockroach,
Periplaneta americana. X 4365.

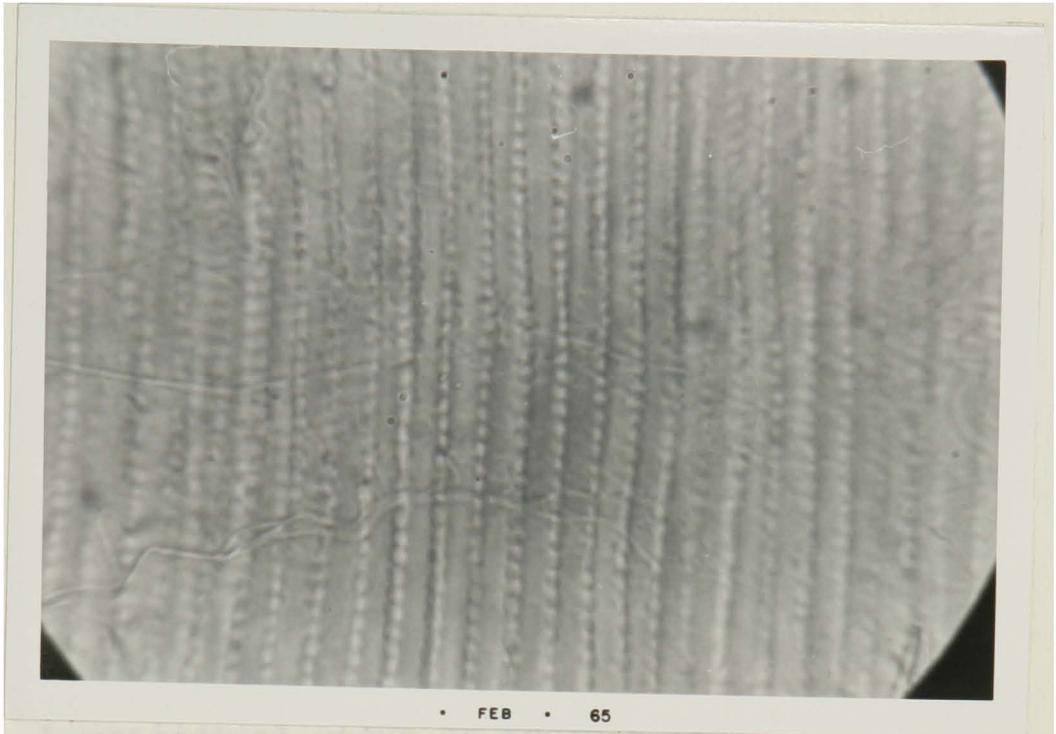


Fig. 2

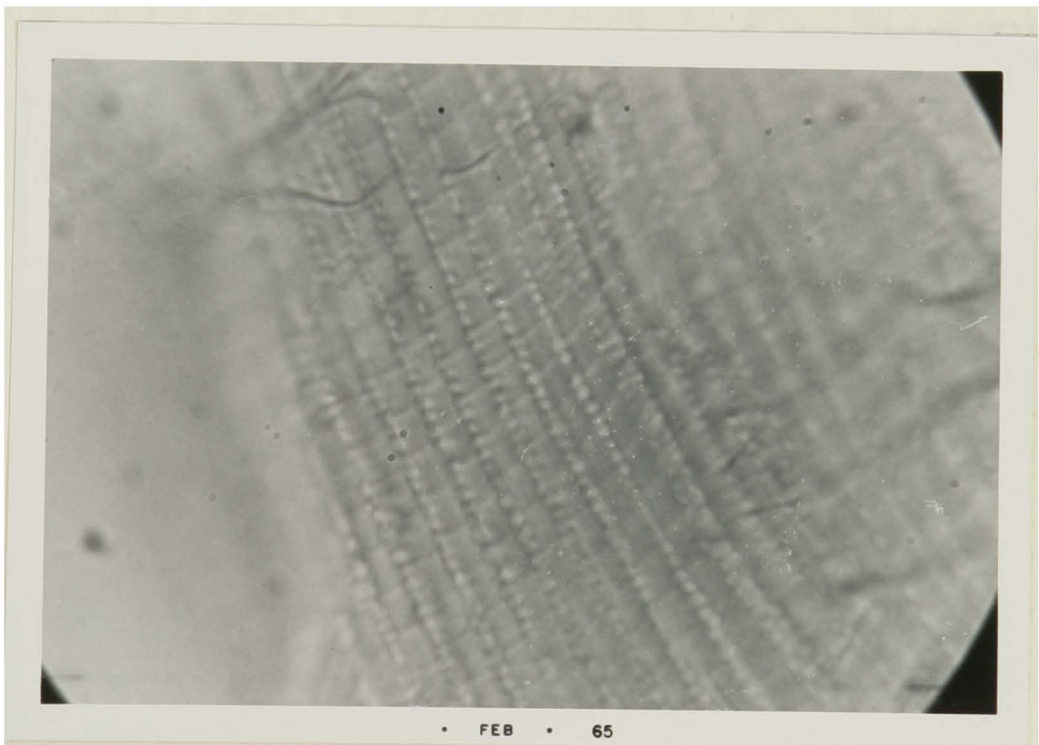


Fig. 3

Explanation of Figures 4 and 5

Longitudinal views of freshly isolated fibers from the Dorsolongitudinal (Fig. 4) and Tergo-sternal (Fig. 5) flight muscles of the dragonfly, Libellula luctuosa. X 5529.

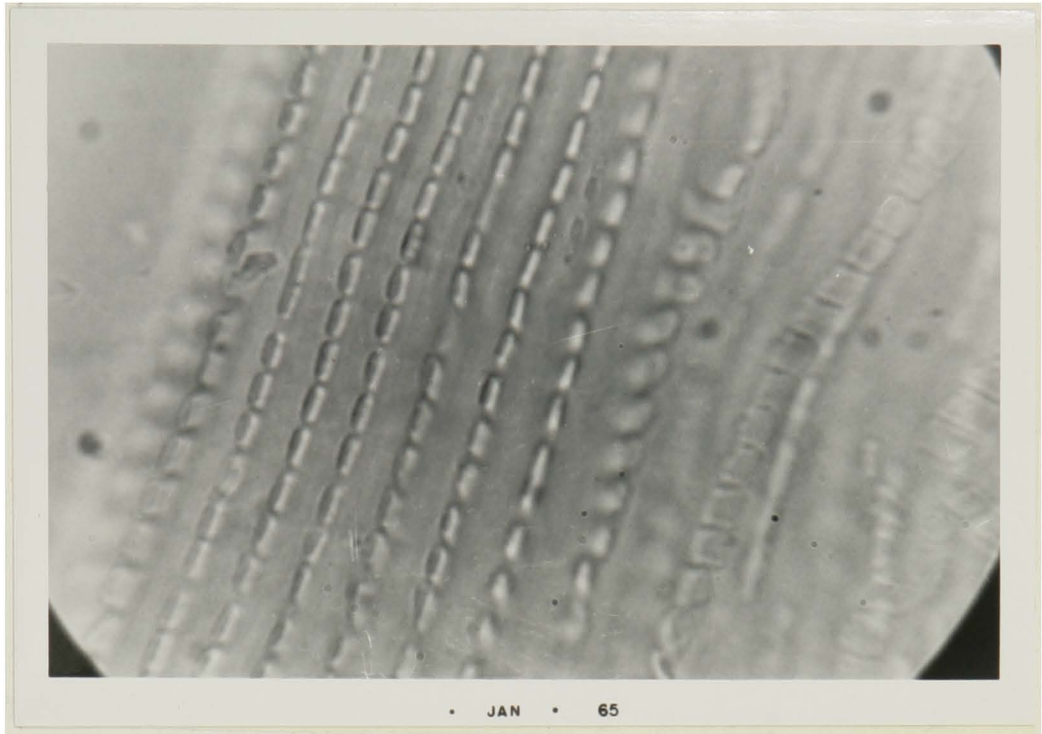


Fig. 4



Fig. 5

Explanation of Figures 6 and 7

Longitudinal views of freshly isolated fibers from the Dorsolongitudinal (Fig. 6) and Tergosternal (Fig. 7) flight muscles of the moth, Protoparce sexta. Fig. 6, X7760; Fig. 7, X3880

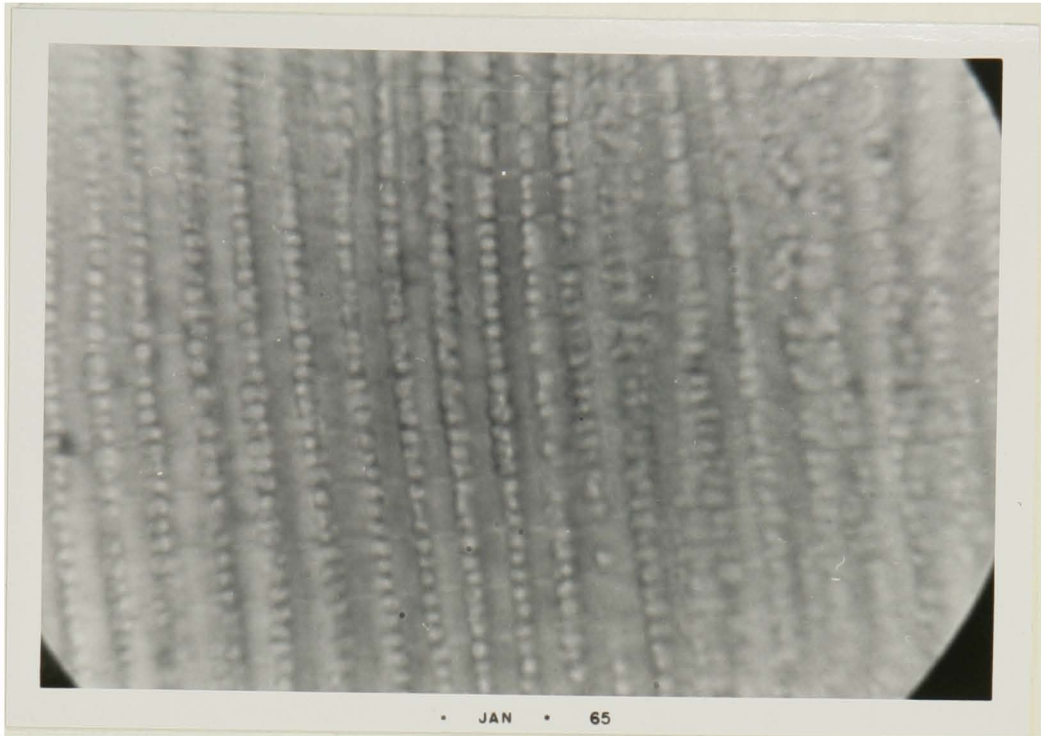


Fig. 6

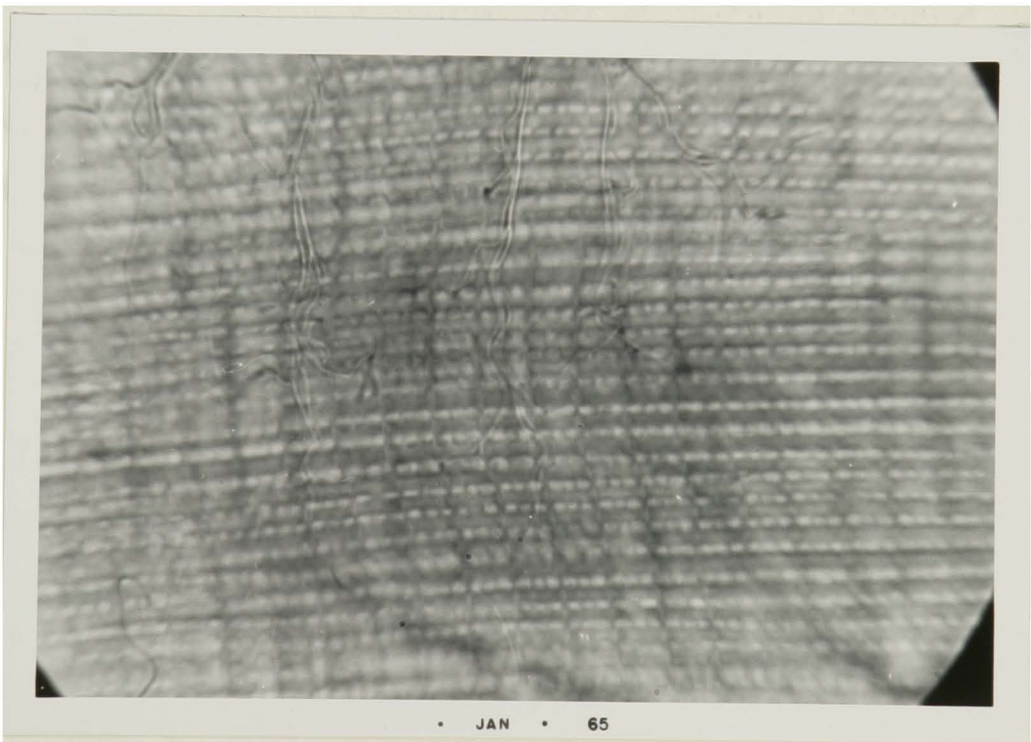


Fig. 7

Explanation of Figures 8 and 9

Longitudinal views of freshly isolated fibers from the Dorsolongitudinal (Fig. 8) and Tergosternal (Fig. 9) flight muscles of the female dobsonfly, Corydalis cornutus. Fig. 8, X7330; Fig. 9, X5500.

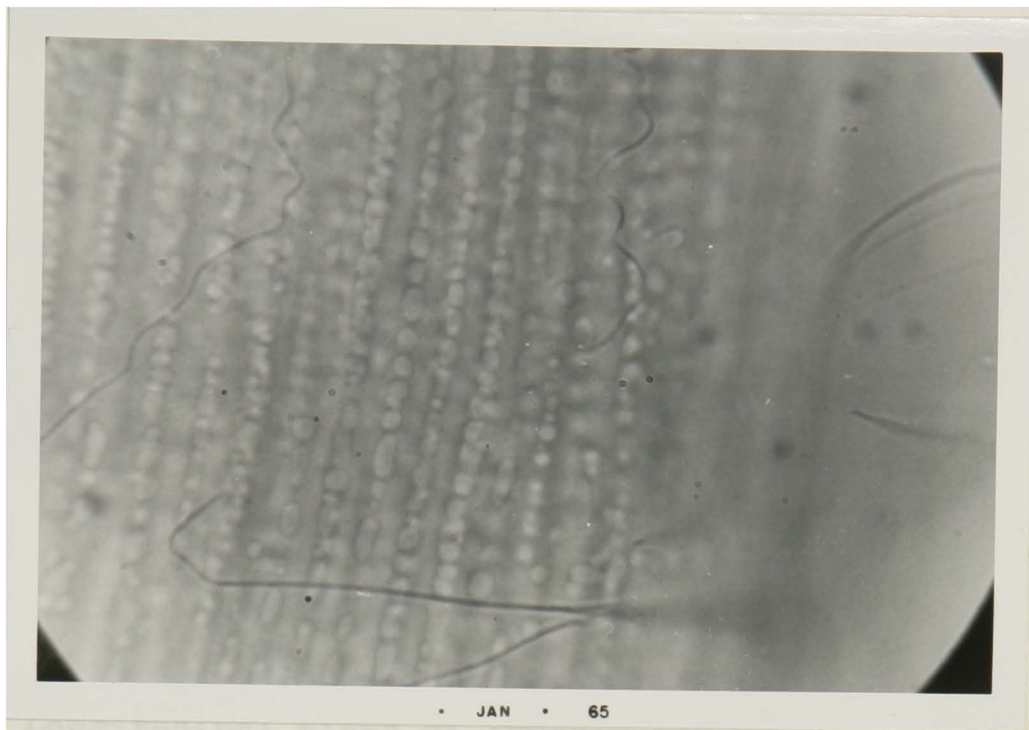


Fig. 8

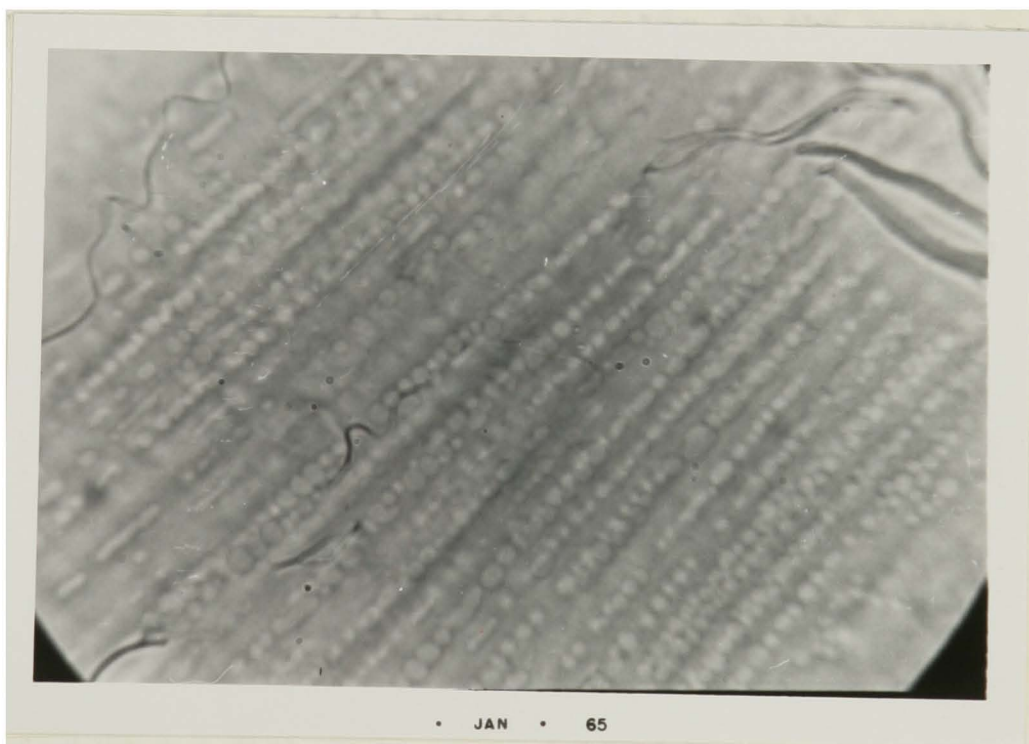


Fig. 9

Explanation of Figures 10 and 11

Longitudinal views of freshly isolated fibers from the Dorsolongitudinal (Fig. 10) and Tergo-
sternal (Fig. 11) flight muscles of the house
fly, Musca domestica. X 7566.



Fig. 10

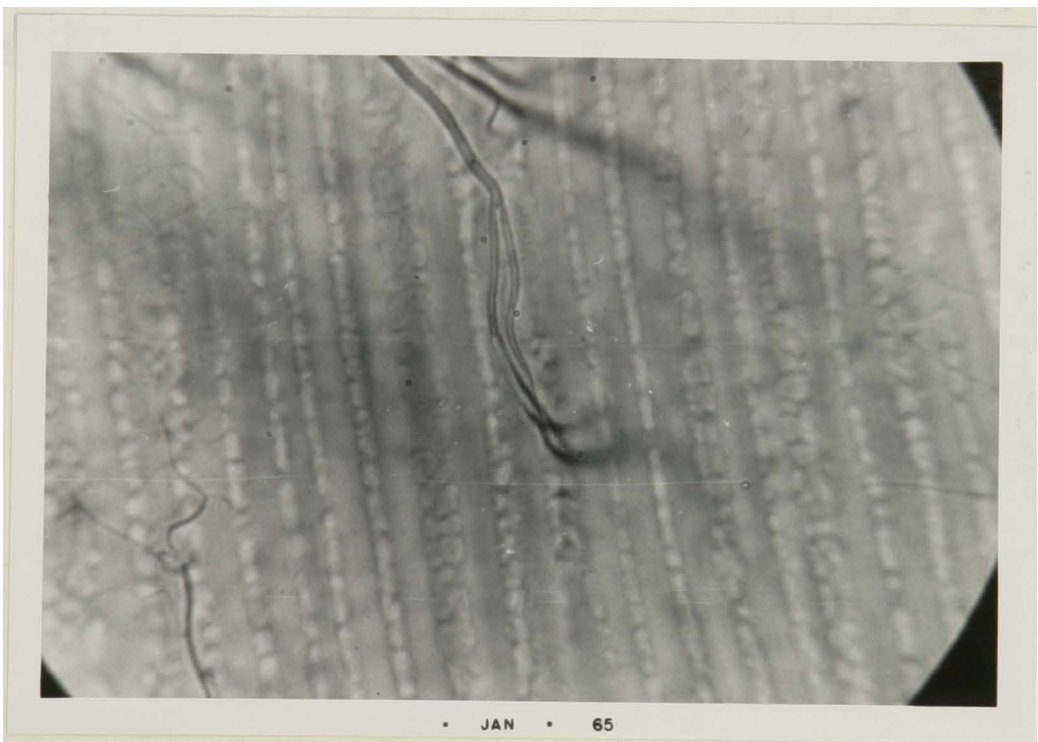


Fig. 11

Explanation of Figures 12 and 13

Longitudinal views of freshly isolated fibers from the Dorsolongitudinal (Fig. 12) and Tergo-sternal (Fig. 13) flight muscles of the face fly, Musca autumnalis. X 5384.

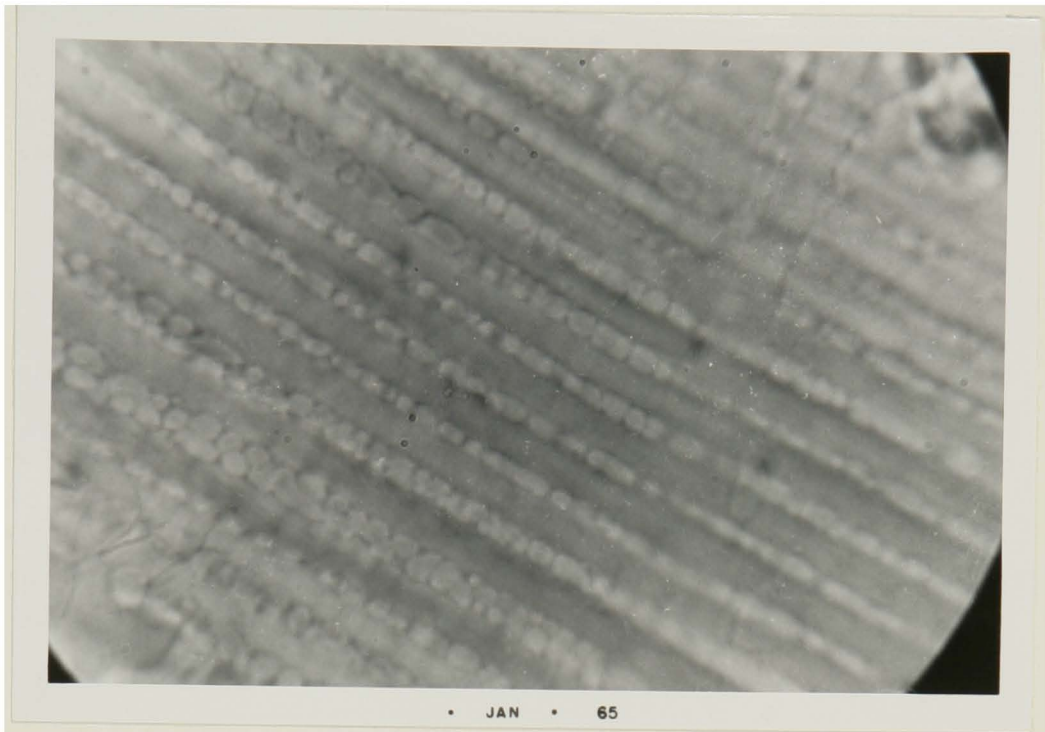


Fig. 12

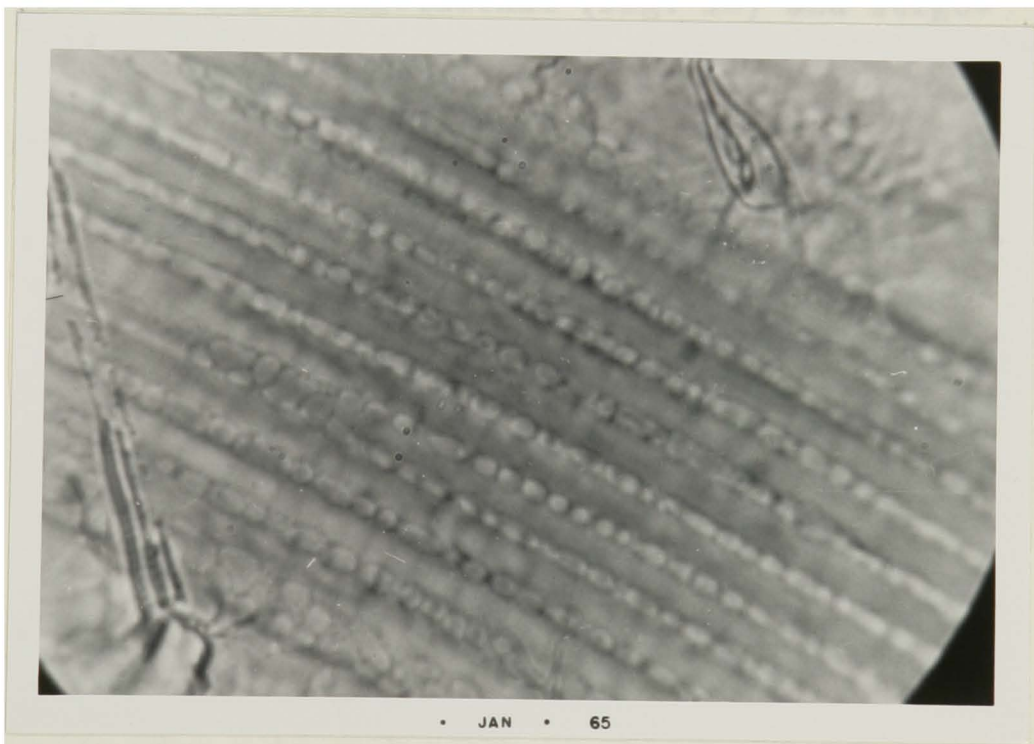


Fig. 13

Explanation of Figures 14 and 15

Longitudinal views of freshly isolated fibers from the Dorsolongitudinal (Fig. 14) and Tergosternal (Fig. 15) flight muscles of the bee, Apis mellifera. Fig. 14, X7566; Fig. 15, X3783.

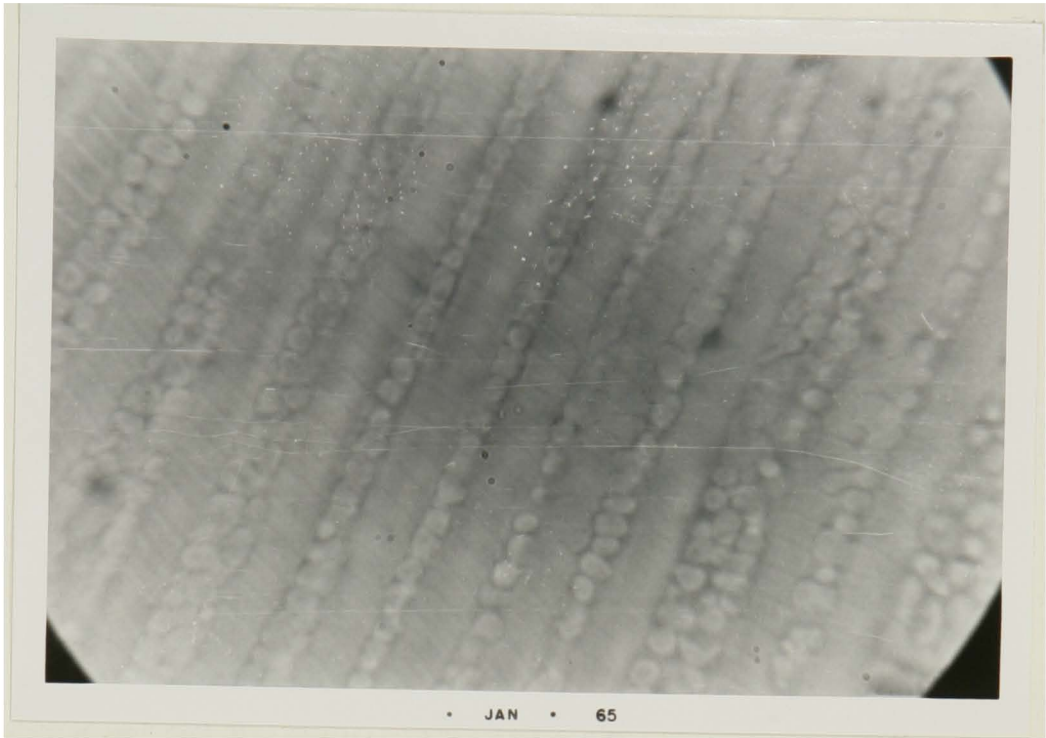


Fig. 14



Fig. 15

Explanation of Figures 16 and 17

Longitudinal views of freshly isolated fibers from the Dorsolongitudinal (Fig. 16) and Tergosternal (Fig. 17) flight muscles of the japanese beetle, Popillia japonica. X7178.

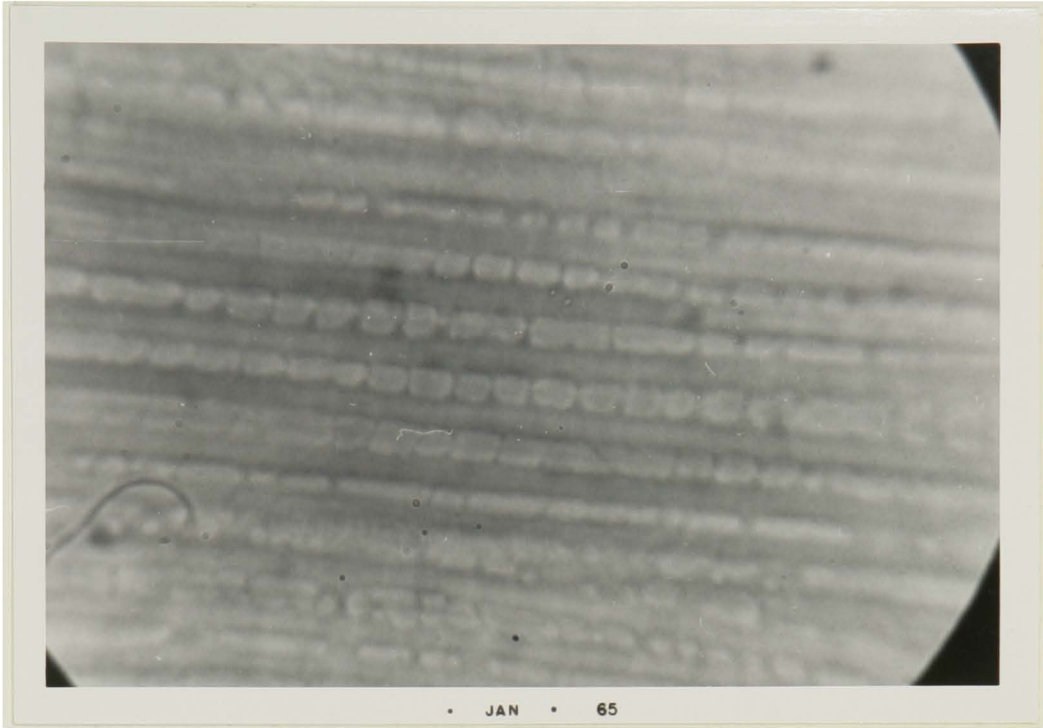


Fig. 16

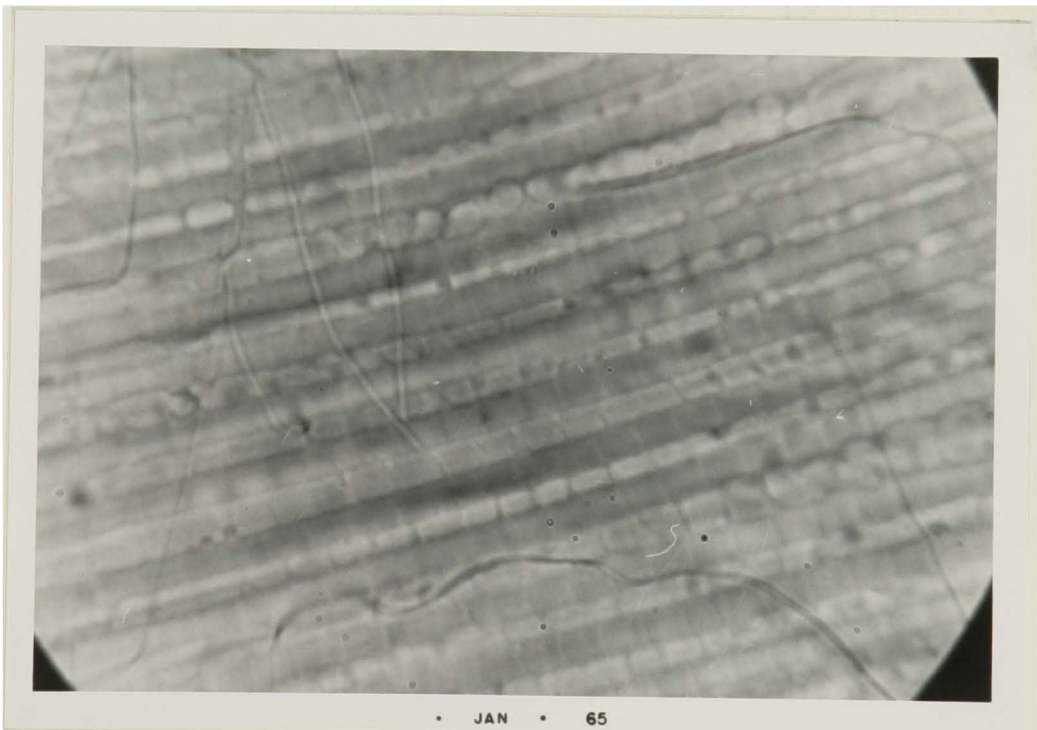


Fig. 17

Explanation of Figures 18 and 19

Longitudinal views of freshly isolated fibers from the Basalar (Fig. 18) and Tergo-pleural (Fig. 19) flight muscles of the female cockroach, Periplaneta americana. Fig. 18, X7372; Fig. 19, X3686.

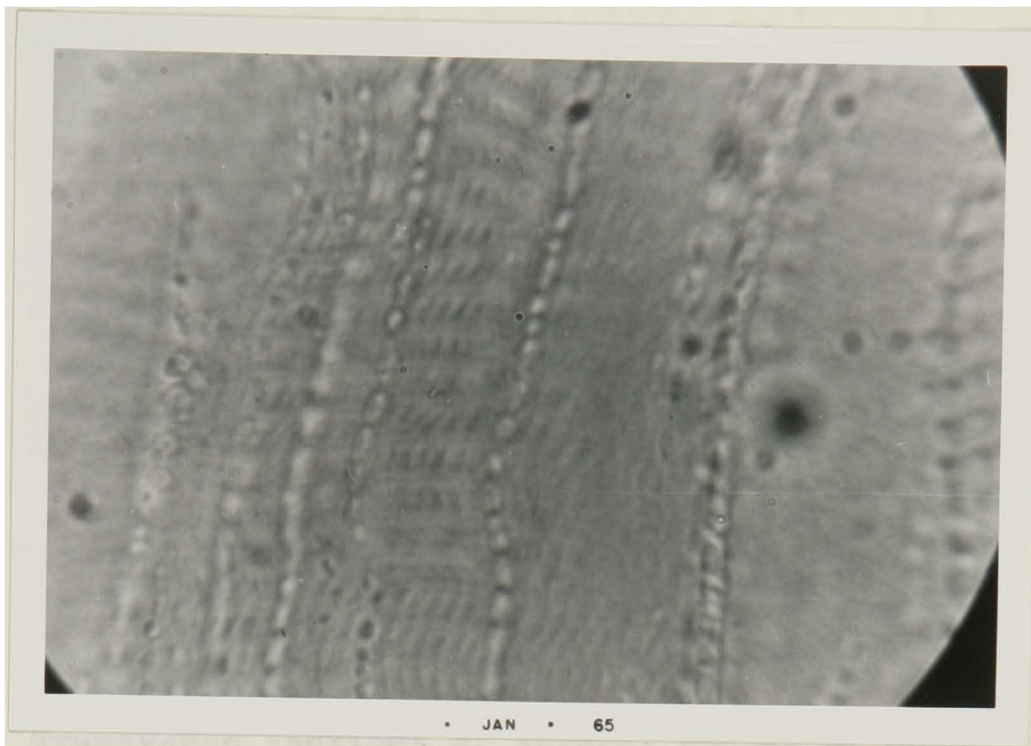


Fig. 18



Fig. 19

Explanation of Figures 20 and 21

Transverse sections of several groups of muscles (Fig. 20) and a close examination of a fiber (Fig. 21) from the complex of the Tergo-pleural flight muscles of the male roach, Periplaneta americana. Fig. 20, X3589; Fig. 21, 7178.

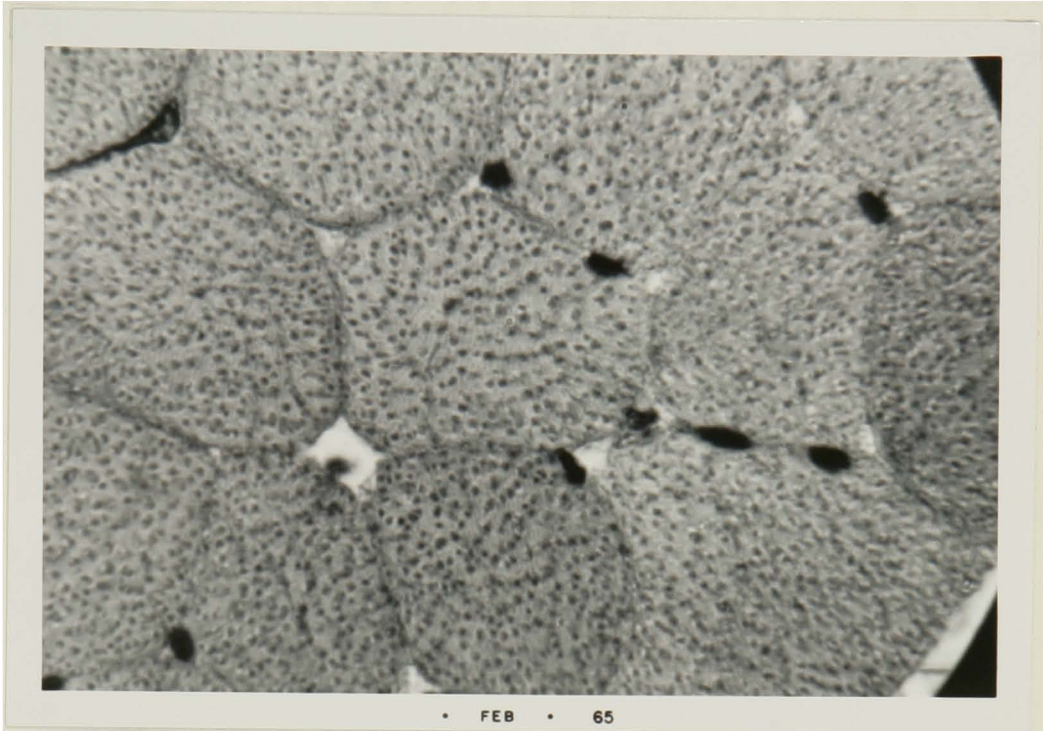


Fig. 20

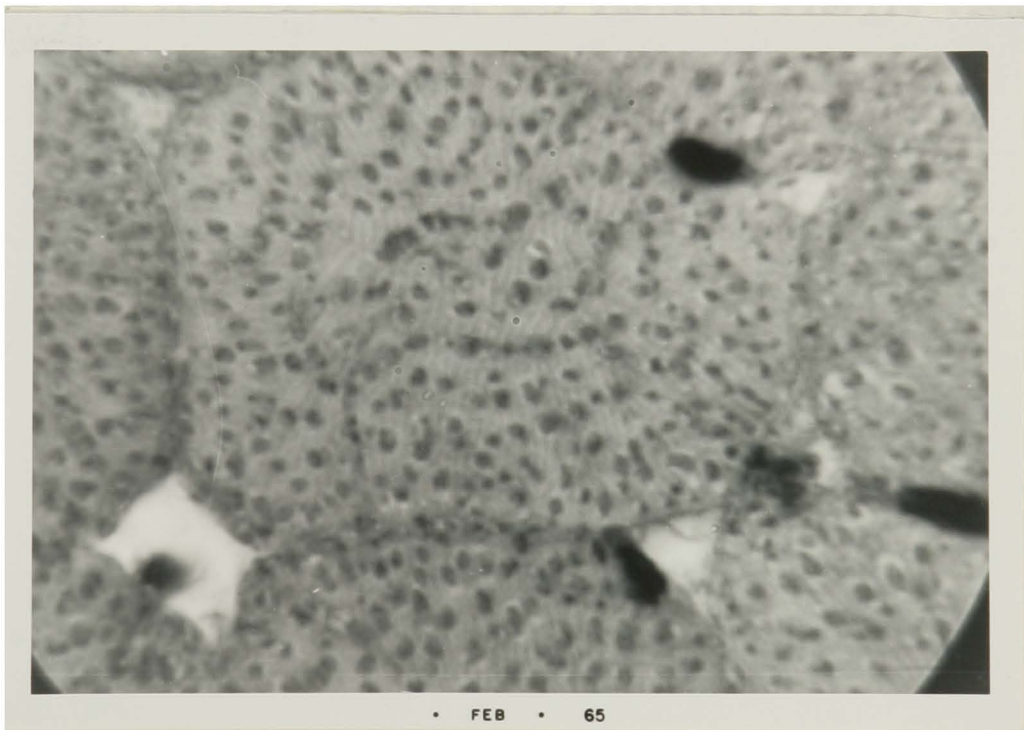


Fig. 21

Explanation to Figures 22 and 23

Drastically (flat fibril in Fig. 22, arrow)
and mildly (thick fibrils, grouped in Fig. 22
and isolated in Fig. 23) isolated fibrils from
fibers of the flight muscles of the male cock-
roach, Periplaneta americana. Fig. 22, X3010;
Fig. 23, X3395.

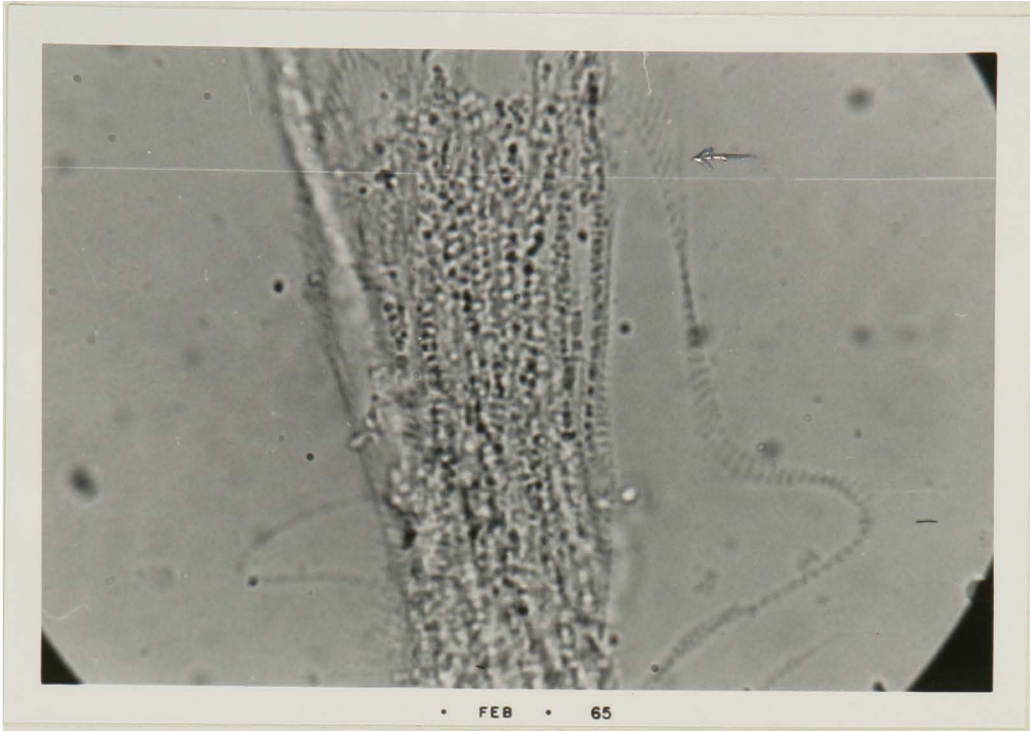


Fig. 22



Fig. 23

Explanation of Figure 24

Transverse section of a fiber from the dorso-ventral flight muscles of the dragonfly, Aeshna sp. X7000.

N = Nucleus

F = Fibril

S = Sarcosome

(Redrawn from Smith, 1961)

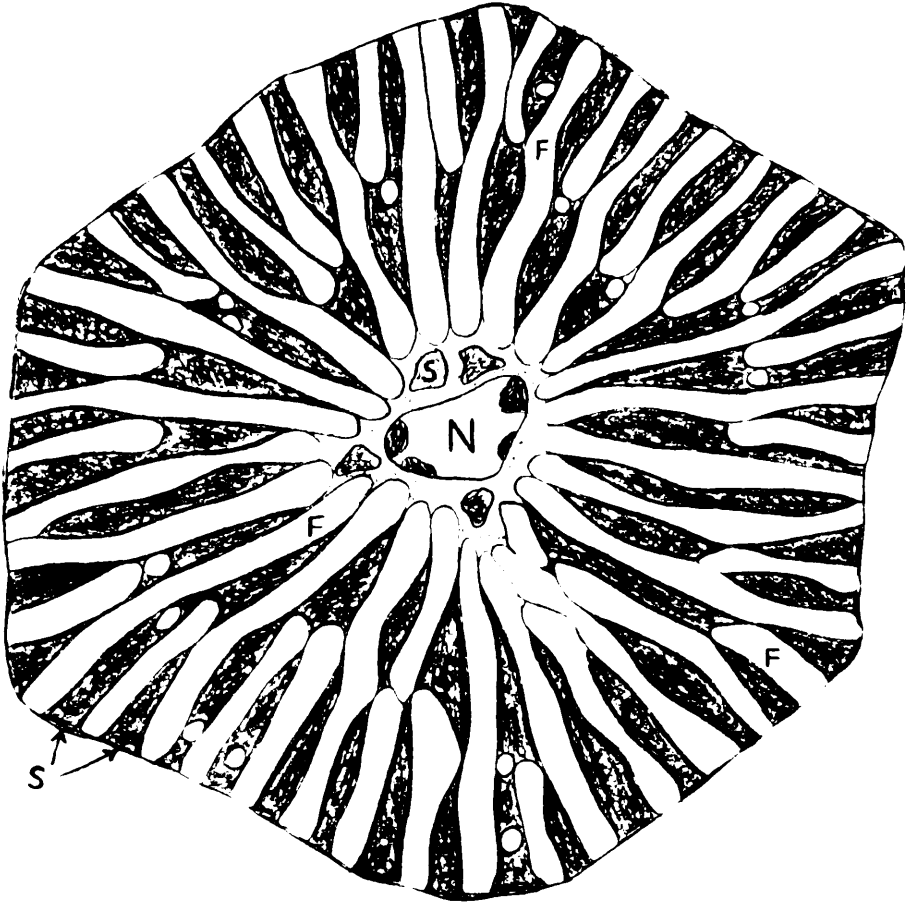


Fig. 24

DISCUSSION

From the evidence presented in this work, it is quite clear that the oxidative capacity of muscle mitochondria from all of the species studied is, in general, comparable. This is true in spite of the fact that several histologically recognizable muscle types are involved, and quantitative differences in rates of oxidation do occur. If the data are examined closely certain considerations become evident and help in our understanding of the results.

The results presented in Table 1 show that when succinate and α -glycerol phosphate were the substrates, high rates of oxidation were achieved with all species. It is true that some variations in rates do exist, but this is to be expected since each species is an individual entity, and the preparation or incubation conditions may not have been optimum for all species. Nevertheless, a remarkable degree of uniformity was present for such a diverse group of insects.

When the DPN-linked substrates are considered, quite a different picture emerges. Here it is only with Periplaneta sarcosomes that high rates of oxidation occur. Sarcosomes from Protoparce and Corydalus gave results which were reasonably good, but not as good as with the roach. All of the other species examined gave results which were much lower.

In searching for an explanation of the results for the DPN-linked substrates, several of the findings reported here appear to be relevant. First, permeability barriers were definitely shown not to play a major role. By several means of mild disintegration it was established that DPN-linked respiration is adversely affected by such treatment. Only when succinate and in some instances α -glycerol phosphate were the substrates was it shown that significant stimulations in respiratory rates occurred. Why this is true is not clear, but it may have a bearing on the finding that succinate and α -glycerol phosphate oxidations are not under the control of ADP.

Second, it appears from the cofactor studies that DPN can stimulate the oxidation of DPN-linked substrates in those species which gave low rates of oxidations with these substrates, but not in Periplaneta sarcosomes. This leads to the speculation that there has been a differential leaking and/or a reduction of the available DPN from the sarcosomes of the different species. Birt (1961) has observed a loss of 15% of DPN in Musca sarcosomes incubated with or without substrates, and a reduction of DPN when these sarcosomes were incubated in the presence of several substrates. The studies on cofactor additions presented here were only preliminary, but there is reason to believe that further work in this area might yield interesting results. This is further

supported by the data in Table 19 which show that the average sarcosome size of the three species (Periplaneta, Protoparce, and Corydalis) giving the best rates of oxidation of DPN-linked substrates is somewhat smaller than the average size of the other species. In fact the average size for Periplaneta sarcosomes is somewhat smaller than those of Protoparce and Corydalis. It is quite possible that the isolation and incubation conditions which were developed specifically for Periplaneta are sufficiently off the optimum conditions for the larger sarcosomes so as to render them susceptible to swelling, leaking, or inactivation of intramitochondrial constituents such as DPN. The different biochemical behavior exhibited by Libellula (Odonata), in contrast to Periplaneta sarcosomes, can also be ascribed to their different sarcosomal size. In Odonata, mitochondria are large and therefore share some of the properties common to the large sarcosomes present in fibrillar fibers. Among the differences which have been observed between small (isolated from tubular muscles) and large (isolated from fibrillar muscles) sarcosomes is the space between their double-membrance structure. There is electron-microscope evidence which indicates that large sarcosomes have a more open internal double-membrane structure than the small sarcosomes (Philpott and Szent Gyorgyi, 1955).

It is on the basis of these findings that the validity of the results obtained by Van den Bergh and Slater (1962) with sonicated sarcosomes from Musca domestica is questioned. In their work glutamate oxidation, as well as that of Krebs cycle intermediates except pyruvate+malate, was notably increased, but if some attention is given to their data the following facts are observed: first, the QO_2 values obtained for succinate and glutamate with untreated sarcosomes were very low (36 and 23, respectively) so their changes in oxidation were dramatic. Second, in the case of glutamate, pyruvate+malate, and other DPN-linked substrates, a cofactor mixture (DPN, TPP, COA, nicotinamide) as well as other compounds (Glutathione) was added.

In the present work, it was found that in Apis sarcosomes DPN caused the stimulation of respiration when pyruvate+malate was oxidized in the presence of a cofactor concentrate. It was also found that if CoA, TPN, or pyridoxal phosphate were added singly to sarcosomes oxidizing pyruvate+malate, oxidation was greatly inhibited. Accepting the possibility that not only these, but other cofactors as well, can inhibit the oxidation of pyruvate+malate in sarcosomes other than Apis we can assume this type of inhibition was probably responsible for the lack of oxidation of this combination of substrates by sonicated mitochondria from Musca in the work of Van den Bergh and Slater (1962). In this

respect, Cochran (1963), working with Periplaneta sarcosomes, showed that among other substrates DPNH is oxidized to about the same extent as some other DPN-linked substrates ($QO_2=18$). Therefore, it is most probable that the stimulation of respiration obtained by Van den Bergh and Slater for some substrates was due in great part to the effect of the cofactor(s) added (cofactors were only added to sonicated sarcosomes), rather than to the sonication treatment by itself.

Although the respiratory rates obtained for the DPN-linked substrates fluctuated widely between species, in all cases the P:O values observed (2.9-3.1) were comparable to those reported for other insects and mammalian systems as well (Lehninger et al., 1958; Sacktor and Cochran, 1958; Cochran and King, 1960; Stegwee and Kammen-Wertheim, 1961; and Cochran, 1963). The same was not true in the case of non-DPN-linked substrates, where the very high QO_2 's obtained with succinate and α -glycerol phosphate in all species, were coupled to different phosphorylating capacities. Of interest here is the fact that the highest P:O ratios were obtained with both substrates by the small sarcosomes isolated from Periplaneta and Protoparce. On the other hand, the lowest P:O values were those corresponding to preparations of large mitochondria (Musca sp and Apis) isolated from fibrillar flight muscles.

The existence of tight respiratory control in sarcosomes isolated from all species tested, except Apis, was only demonstrated for the oxidation of the DPN-linked substrates, glutamate, pyruvate+malate, and α -ketoglutaric acid. This finding confirms similar observations previously reported by Klingenberg and Bucher (1959) with Locusta, Gregg et al. (1960) with Musca, Stegwee and Kammen-Wertheim (1961) with Leptinotarsa, Musca, Periplaneta, and Locusta, Birt (1961) with Musca and Lucilia, and Cochran (1963) with Periplaneta, but deviates from those of Van den Bergh and Slater (1962) with Musca, who stated that glutamic acid and other Krebs cycle intermediates are oxidized poorly, with a poor phosphorylation, and no measurable respiratory control.

In the case of Apis mellifera, respiratory control was not demonstrated in the oxidation of pyruvate+malate, since comparable QO_2 are obtained in the presence or absence of ADP. This characteristic of Apis mitochondria was not observed for any other preparation in this study but the true significance of this finding is not known.

On the other hand, a lack of respiratory control is obvious in the oxidation of succinate and α -glycerol phosphate by sarcosomes from all sources investigated. Both of these substrates gave in all cases comparable

QO_2 with or without ADP. Similar observations for succinate have been reported by Birt (1961) with Musca. Previous results by Sacktor and Cochran (1958) with Musca, Cochran and King (1960), and Cochran (1963) with Periplaneta, support the present findings for α -glycerol phosphate. Other workers are in disagreement, and state that ADP does have a stimulatory effect on the oxidation of this substrate (Bucher et al. (1959) with Locusta, Gregg et al. (1961), Birt (1961), and Van den Bergh and Slater (1962) with Musca, Sacktor and Packer (1961), with Phormia, and Stegwee and Kammen-Wertheim (1962) with Leptinotarsa, Musca, Periplaneta, and Locusta. Cochran (1963), working with Periplaneta sarcosomes, reported that the oxidation of succinate in the absence of ADP was more than twice that obtained in the presence of this nucleotide. In that paper he also reported, in agreement with the work of Birt (1961), that the protein concentration of the reaction medium does exert an influence in the response produced by ADP in these oxidations. He found that at concentrations of 3.5 mg. of protein per flask or higher, the stimulatory influence of ADP might easily be missed. Since in our mitochondrial preparations the amount of protein per flask was always between 3.6 and 4.8 mg, it is reasonable to suppose that at these high protein concentrations the influence of ADP on succinate and α -glycerol phosphate was masked. Thus, in both substrates, comparable QO_2 were obtained with or without ADP.

An explanation for the different control of respiration in sarcosomes oxidizing DPN-linked and non-DPN-linked substrates observed in this investigation, when ADP was absent, might be as follows:

It has been established that an inhibitory substance responsible for respiratory control is present in the mitochondria in amounts which are very similar to their Pyridine nucleotide content, Chance (1958). This intramitochondrial substance has been found to be a bound form of reduced Pyridine nucleotide, which is extremely labile in the presence of substances which in one way or another modify the mitochondrial structure (Chance and Conrad, 1958; Chance and Baltscheffsky, 1958). The reduction of this form of DPN is greater in the absence of ADP and phosphate (Chance, 1959), and/or when sarcosomes are incubated in the presence of succinate, pyruvate+malate, and to a minor extent by α -glycerol phosphate (Birt, 1961).

On this basis it is possible to explain that in our case a probable reduction or inactivation of this labile intramitochondrial bound-DPN took place with mild disintegration treatments, incubation with succinate, α -glycerol phosphate, pyruvate+malate, and in the absence of ADP. Accordingly, this effect was adverse only in the oxidation of the DPN-linked substrates where this nucleotide is required as the first step of the respiratory chain. On the other hand, succinate and α -glycerol

phosphate, which are non-DPN linked substrates, were not affected (but in some cases stimulated) by any of the factors known to determine the availability of intramitochondrial DPN, such as the case in which ADP was absent. Succinate oxidation was probably increased with short term sonications due to a decrease of functional mitochondrial protein (enzymes) in the sonicate. This is true since prolonged or repeated treatments gradually lowered the O_2 consumption of the preparation, indicating that sound at this duration probably inactivated or damaged the normal chain of enzymes (proteins) in the respiratory chain after the more vulnerable DPN step.

Contrary to report by Sacktor (1954), Sacktor et al. (1958), and Wojtczak and Wojtczak (1959, 1960), but in agreement with results obtained by Van den Bergh and Slater (1962) and Cochran (1961), evidence is presented in this work that the presence of serum albumin in the preparation and/or reaction media is not essential to obtain maximal respiratory rates with all substrates tested when oxidized by isolated mitochondria from Periplaneta and Musca. When mitochondrial preparations from these species are treated in this manner and later subjected to a sonic treatment, however, the stimulatory effect of short sonication times on the oxidation of succinate by untreated or KCl-EDTA isolated sarcosomes was no longer observed. These results indicate that

although serum albumin is not essential in the respiration of these sarcosomes, it does confer a certain degree of protection to them by further stabilizing their structure.

Although in early investigations the absence of sarcosomes in the flight muscles of male and female Periplaneta was stated by Edwards et al. (1954), it became clear in the present study that not only in this species but in all insects investigated the occurrence of numerous and highly organized sarcosomes of variable shapes and sizes was a general rule. Since in this work the histological material included specimens which contained one or another of the commonly recognized three main types of flight muscles, it can be assumed that the relative abundance of mitochondria and their characteristic arrangement is the same in the pigmented flight muscles of all insect species.

In the case of the white flight muscles of female Periplaneta, the sarcosome arrangement also occurs but the number of mitochondrial rows per fiber is greatly reduced. Therefore, since the reddish coloration of muscle is, for the most part, due to the high cytochrome content of the mitochondria this considerable reduction in the sarcosome number can account for the absence of visible pigmentation characteristic of the female roach musculature.

The histological differentiation of the fibers isolated from the flight muscles of Periplaneta is only seen in a cross-section slide preparation which has been carefully and selectively stained for this purpose. Once a good slide preparation is obtained a subcellular observation (contrary to a statement by Pipa, 1955) of the distribution of the contractile material becomes possible even with a standard light microscope.

The microscopic observations of the cross-sections of the flight muscle fibers of male Periplaneta indicated, in disagreement with the views of several authors (Pringle, 1957; Pipa, 1955; Smith, 1961; and Howell, 1963), that in this species there are basic differences in the arrangement of its fibrillar components when compared to those of the representative tubular-lamellar fibers of all the flight muscles in Odonata. In the roach muscle cells (muscle fibers) nuclei are scattered at random around the body of the fiber and are commonly observed just beneath the sarcolemma. The term "pseudolamellar" is proposed to describe the very typical fibrillar arrangement of the pigmented flight muscles of male Periplaneta. These independent fibrils are small in diameter, roughly cylindrical to oval in form, and are secondarily organized into composite lamellae. Similar observations for this species were made in several early studies by Keilich (1918), Jordan (1920),

Marcus (1921), and Tiegs 1955, but as indicated earlier most modern investigators maintain that the roach flight muscles are tubular-lamellar and that their contractile material is distributed in flat and large lamellae. Pipa (1955), stated that these flat lamellae in Periplaneta americana are only seen in the electron-micrographs shown by Edwards et al. (1954), but they presented only longitudinal views of single fibrils where, for obvious reasons, their true morphology and dimensional characteristics can only be assumed. That this is true, we can deduce from Edwards' explanation for the apparent flat lamellar shape of the tubular fibrils. From the present work it is concluded that the following factors may be of importance in determining the flat form of these fibrils:

1. A defective preparation of the fibrillar material under observation.
2. High water content of Tubular fibrils (which makes them susceptible to be deformed from their original shape).
3. Difference in sarcolemma thickness (thicker in the white muscles) or the arrangement of forces within the muscle.

In agreement with these points, evidence is given in Figs. 20, 21 and 22, 23 which shows the difference in shape that a fibril takes when observed separated from its normal location, and its roughly cylindrical form when observed in situ.

SUMMARY AND CONCLUSIONS

Comparative histological and biochemical studies were conducted in an effort to determine the functional relationship which exists between the subcellular organization and biochemical activity of flight muscles from several insect species. For this purpose, representative species which contained the three main types of muscle fibers were selected.

The respiratory activity of sarcosomes isolated from the thoraces of all species was measured manometrically in a conventional Warburg respirometer at 25°C. The importance of using a standard isolation, preparation, and reaction media, as well as standard techniques in the preparation and chemical evaluation of all mitochondrial suspensions was recognized in this work. These experimental conditions may very well be essential in the validity of comparative results in studies of this nature.

Results obtained from biochemical and histological observations on the flight muscles of the test animals can be summarized as follows:

1. Mitochondria isolated from each of the test species metabolized the substrates used in this study at varying rates. The highest respiratory rates were obtained with the non-DPN-linked substrates α -glycerol phosphate and succinate (except in Periplaneta sarcosomes

where succinate had the lowest QO_2 for that species). They were followed by the DPN-linked substrates pyruvate+malate, α -ketoglutarate and glutamate (except with Periplaneta sarcosomes which oxidized glutamate at very high rates).

2. When the oxidative efficiency of sarcosomes was compared between species, it was found that with all substrates tested the highest QO_2 values (except for succinate) were obtained with sarcosomes from Periplaneta, while the second and third highest QO_2 values were those of Protoparce and Corydalus, respectively. The lowest QO_2 's for all substrates, were those obtained with mitochondria isolated from all species with fibrillar flight muscles and from Odonata. Therefore, it appears from these results that not only the fibril size, but also the size of the mitochondria are important in the characterization of the metabolic activities of the animal.

3. Although the respiratory rates obtained with all substrates and by all species fluctuated widely, P:O values comparable to those found by other authors in insect and mammalian systems were observed.

4. A tight respiratory control by phosphate acceptor (ADP) was demonstrated in all species for the oxidation of DPN-linked substrates (glutamic acid, pyruvate+malate and α -ketoglutarate), but this was not the case for the non-DPN-linked substrates (succinate and α -glycerol

phosphate). With the latter substrates maximal respiratory rates could be obtained with or without this nucleotide. Respiratory control could not be demonstrated in sarcosomes isolated from Apis, when pyruvate+malate were the substrates being oxidized. The probable factors involved in the outcome of respiratory control in sarcosomes oxidizing these two types of substrates were discussed, and their effect on respiration was suggested.

5. The existence and importance of permeability barriers in active sarcosomes towards the oxidation of DPN-linked substrates, was investigated by several means of mild disintegration. In all cases permeability barriers were shown not to play an important role in limiting the rate of oxidation. Only in the case of succinate, and in some instances (freezing and thawing) α -glycerol phosphate, was it shown that a stimulation of respiration occurred as a result of these treatments. An explanation for these results was tentatively suggested.

6. The suggestion was made that the dramatic stimulation in the oxidation of some DPN-linked substrates, after an ultrasonic treatment observed by some authors (Van den Bergh and Slater, 1962), could be due, to a large extent, to the effect of cofactor additions rather than to the net effect of the sonic treatment itself. In support of this view results are presented

which indicate that the oxidation of pyruvate+malate and glutamate by intact (untreated) Apis sarcosomes could be stimulated considerably by adding a cofactor mixture plus Glutathione in the case of glutamate) to the standard reaction medium.

7. In experiments where the stimulatory effect of single coenzymes added to a medium containing Apis sarcosomes was studied, it was found that DPN was the only cofactor causing a stimulation comparable to the stimulation caused by the total coenzyme mixture. Furthermore, when pyridoxal phosphate (vit. B₆), CoA or TPN were added singly they actually inhibited respiration. The effect of these coenzymes is also substrate specific, since succinate oxidation was not affected by them, while pyruvate+malate and glutamate oxidations were stimulated.

8. The cofactor stimulatory effect observed in Apis sarcosomes, was not observed in sarcosomes from Periplaneta.

9. The presence of serum albumin in both the preparation and incubation media was not an essential requirement to obtain maximal QO₂ values for all substrates with sarcosomes from Periplaneta or Musca autumnalis. It was observed, however, that this protein conferred a certain degree of protection in all treated sarcosomes.

10. Short preincubation periods in a deoxycholesterol medium (in the cold), greatly reduced the oxidative capacity of Periplaneta sarcosomes.

11. The fibril and sarcosomes sizes (in diameter or thickness) were found to be smaller in the "pseudo-lamellar" muscles of Periplaneta than those in the "microfibrillar" and "fibrillar" muscles studied.

12. The results of micro-histological studies of isolated flight muscles from Periplaneta showed that muscle fibers from this insect are composed of small and roughly cylindrical to oval-shaped (when in situ) fibrils which are secondarily arranged into a lamellar-like structure. To describe these specific fibers, the term "pseudo-lamellar" fibers was suggested to avoid grouping this type of fiber together with the "tubular" fibers typical of Odonata.

ACKNOWLEDGEMENTS

The author is greatly indebted to Dr. D. G. Cochran under whose supervision this work was carried out, and whose suggestions, advice and cooperation made this work possible.

Special thanks are extended to Dr. J. McD. Grayson, Head, Department of Entomology, V.P.I., for his cooperation, encouragement and his continuous assistance in many ways during the author's graduate studies.

The writer wishes to express his gratitude to Drs. J. L. Bishop, E. C. Turner, Jr., R. E. Benoit, N. R. Krieg, M. G. Hale and R. R. Mills, for their technical advice and suggestions during the writing of this manuscript.

Sincere thanks are also extended to Dr. R. B. Holliman and Mr. J. H. Roles, Jr., for help in the preparation and staining of the transverse-sections of the flight muscles of the cockroach, Periplaneta americana, which was important in one of the histological phases of this research.

Gratitude is expressed to Mr. J. M. Amos for assistance in providing the honey bees used in these experiments.

The writer offers his appreciation to his fellow students at V.P.I. for assistance and cooperation in a

number of ways; especially to Mrs. Jan Fuller de Pamanes for her great interest and unselfish help in certain phases of this research.

The author wishes to express his deep and sincere gratitude to his mother, Tonita, without whose sacrifice, patience and moral assistance this project could not have been completed.

Sincere and special thanks are due to the author's wife, Olguita for her patience, moral assistance and outstanding understanding throughout my undergraduate and graduate studies.

BIBLIOGRAPHY

1. Aubert, H. (1853) Referred to in Dr. Tiegs paper. Tiegs, O. W. (1955) The flight muscles of insects - their anatomy and histology; with some observations on the structure of striated muscle in general. Phil. Trans. B 238, 221-359.
2. Boettiger, E. G. and Furshpan, E (1952) The mechanics of flight movements in Diptera. Biol. Bull. 102, 200-211.
3. Boettiger, E. G. (1957) The machinery of insect flight. In Recent Advances in Invertebrate Physiology, (B.T. Scheer, editor), Univ. of Oregon Publications, 117-42.
4. Boettiger, E. G. (1958) The effect of length change on the tension-sustaining ability of the Basalar muscle of the rhinoceros beetle. Anat. Record, 132, 418.
5. Boettiger, E. G. (1960) Insects flight muscles and their basic physiology. Ann. Rev. Entomol., 5, 1-15.
6. Birt, L. M. (1961) Flight muscle mitochondria of Lucilia cuprina and Musca domestica. Estimation of the pyridine nucleotide content and of the response of respiration to adenosine diphosphate. Biochem. J , 80, 623.
7. Bucher, T. and Klingenberg, M. (1958) Wege des Wasserstoffs in der lebendigen Organisation. Angew. Chem. 70, 552.
8. Bucher, Th., Klingenberg, M., and Zebe, E. (1959) Discussion of Dr. Sacktor's paper. Proc. 4th Int. Congr. Biochem., Vienna, 12, 153-160.
9. Chadwick, L. E. (1953) The motion of the wings. Aerodynamics and flight metabolism. In Insect Physiology, (K. D. Roeder, Ed.), J. Wiley and Sons, Inc., New York, N. Y.
10. Chance, B. (1952) Spectra and reaction kinetics of respiratory pigments of homogenized and intact cells. Nature, 169, 215-221.
11. Chance, B. (1954) Spectrophotometry of intracellular respiratory pigments. Science, 120, 767-775.

12. Chance, B. (1956) On possible mechanisms for the control of electron transport in the respiratory chain. Proc. 3th Int. Congr. Biochem., New York, (Academic Press), 300.
13. Chance, B. and Baltscheffsky, H. (1958) Respiratory enzymes in oxidative phosphorylation. J. Biol. Chem., 233, 736-739.
14. Chance, B. and Conrad, H. (1958) Intramitochondrial Pyridine nucleotide. Fed. Proc. 17, 200.
15. Chance, B. and Sacktor, B. (1958) Respiratory metabolism of insect flight muscle. II Kinetics of respiratory enzymes in flight muscle sarcosomes Arch. Biochem. Biophys., 76, 509.
16. Chance, B. (1959) Quantitative aspects of the control of Oxygen utilization. Ciba Foundation Symposium on the Regulation of Cell Metabolism, (Ed. J. S. A. Churchill Ltd.) London, 91-121.
17. Cochran, D. G. (1961) The influence of serum albumin on oxidative phosphorylation in mitochondria from cockroach muscle. Proc. 11th Intern. Congr. Entomol., Wien, 1960 I, 663-667.
18. Cochran, D. G. (1963) Respiratory control in cockroach-muscle mitochondria. Biochim. Biophys. Acta 78, 393-403.
19. Edwards, G. A., Santos P. de S., Santos H. L. de S., and Sawaya, P. (1954) Electron microscope studies of insect muscle. II Flight and leg muscles of *Belostoma* and *Periplaneta*. Ann. Entomol. Soc. Amer. 47, 459-467.
20. Estabrook, R. W. and Sacktor, B. (1958) a-Glycerol phosphate oxidase of flight muscle mitochondria. J. Biol. Chem., 233, 1014-1019.
21. Fiske, C. H. and SubbaRow, Y. (1925) The colorimetric determination of phosphorus. J. Biol. Chem., 66, 375-400.
22. Gonda, O., Traub, A., and Avi-Dor, Y. (1957) The oxidative activity of particulate fractions from mosquitoes. Biochem. J., 67, 487-493.

23. Gregg, C. T., Heisler, C. R. and Remmert, L. F. (1960) Oxidative phosphorylation and respiratory control in housefly mitochondria. *Biochim. Biophys. Acta.*, 45, 561-570.
24. Hanson, J. (1952) Changes in the cross-striation of myofibrils during contraction induced by Adenosine triphosphate. *Nature*, 169, 530-533.
25. Howell, V. D. (1963) Close-packed and fibrillar muscles of the Hymenoptera. *Ann. Entomol. Soc. Amer.*, 56, 295-306.
26. Hoskins, D. D., Cheldelin, V. H., and Newburgh, R. W. (1956) Oxidative enzymes systems of the honey bee, *Apis mellifera* L. *J. Gen. Physiol*, 39, 705-713.
27. Ito, T. and Horie, Y. (1959) Further studies on the oxidative enzymes of the midgut of the silkworm, *Bombyx mori*. *Bull. of the sericul. exp. stat.*, 15, 337-353.
28. Jordan, H. E. (1920) Studies on striped muscle structure. VI The comparative histology at the leg and wing muscle structure of the wasp, with special reference to the phenomenon of stripe reversal during contraction and to the genetic relation between contraction bands and intercalated discs. *Amer. J. Anat.*, 27, 1-66.
29. Keilich, J. (1918) Beitrage zur Kenntnis der Insectenmuskeln. *Zool. Jahrb. Anat., u Ontog.*, 40, 515-536.
30. Keilin, D. (1925) On cytochrome, a respiratory pigment, common to animals, yeasts, and higher plants *Proc. Roy. Soc. (London)*, B. 98, 312.
31. Klingenberg, M. and Bucher, T. (1959) Flugmuskel-mitochondrien aus *Locusta migratoria* mit Atmungskontrolle *Biochem. Z.* 331, 312-333.
32. Lehninger, A. L., Wadkins, C. L., Cooper, C. Devlin, T. M. and Gamble, J. L. (1958) Oxidative phosphorylation. *Science*, 128, 450-456.
33. Lester and Crane (1959) The natural occurrence of coenzyme Q and related compounds. *J. Biol. Chem.*, 234, 2169-2175.
34. Levenbook, L. and Williams, C. M. (1956) Mitochondrial cytochrome c in relation to the ageing and wing beat frequency of flies. *J. Gen. Physiol*, 39, 497-512.

35. Lewis, S. E. and Slater, E. C. (1954) Oxidative phosphorylation in insect sarcosomes. *Biochem. J.*, 58, 207.
36. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall (1951) Protein measurement with the Folin-Wu Phenol Reagent. *J. Biol. Chem.*, 193, 265.
37. Marcus, H. (1921) Referred to in Dr. Tiegs paper. Tiegs, O. W. (1955) The flight muscles of insects- Their anatomy and histology; with some observations on the structure of striated muscle in general. *Phil. Trans. B*, 238, 221-359.
38. Philpott, D. E. and Szent-Gyorgyi A. (1955) Observations on the electron microscope structure of insect muscle. *Biochim. Biophys. Acta* 18, 177-182.
39. Pipa, R. L. (1955) A comparative histological study of the indirect flight muscles of various insect orders. Master's Thesis, Uni. of Connecticut, Storrs.
40. Pringle, J. W. S. (1957) In *Insect Flight*, Cambridge Monographs in Experimental Biology, No. 9, Cambridge, Uni. Press, ch. 3.
41. Roeder, K. D. (1951) Movements of the thorax and potential changes in the thoracic muscles of insects during flight. *Biol. Bull.*, 100, 95-106.
42. Sacktor, B. (1952) The Cytochrome c Oxidase of the House Fly, Musca domestica (L.) *J. Gen. Physiol.* 35, 397-407.
43. Sacktor, B. (1954) Investigations on the mitochondria of the house fly, Musca domestica L. III Requirements for oxidative phosphorylation. *J. Gen. Physiol.* 37, 343.
44. Sacktor, B., O'Neill, J. J. and Cochran, D. G. (1958) The requirement for serum albumin in oxidative phosphorylation of flight muscle mitochondria. *J. Biol. Chem.* 233, 1233.
45. Sacktor, B. and Cochran, D. G. (1958) The respiratory metabolism of insect flight muscle. I. Manometric studies of oxidation and concomitant phosphorylation with sarcosomes. *Arch. Biochem. Biophys.* 74, 266-267.

46. Sacktor, B. (1958) A biochemical basis of flight muscle activity. Proc. 4th Intern. Congr. Biochem. Vienna. 12, 138-152.
47. Sacktor, B. (1959) The α -glycerophosphate pathway of electron transfer in insect flight muscle. Ann. Meeting Amer. Chem. Soc., Boston, Mass., Abstr. of papers 30c.
48. Sacktor, B. (1961) The role of mitochondria in respiratory metabolism of flight muscle. Ann. Rev. Entomol. 6, 103-130.
49. Sacktor, B. and Packer, L. (1961) The stimulation of α -glycerophosphate oxidation by adenosine diphosphate in teased flight muscle. Biochim. Biophys. Acta. 49, 402-403.
50. Sacktor, B. (1964) Metabolic significance of the α -glycerophosphate shuttle in skeletal muscle. Proc. 6th Int. Congr. Biochem., New York, Abstract Vol.
51. Siebold, C. T. von (1848) Lehrbuch der vergleichenden Anatomie der wirbellosen Tiere. Berlin.
52. Snodgrass, R. E. (1927) Morphology and mechanism of the insect thorax. Smithsn. misc. Coll., 80, 1-108.
53. Smith, D. S. (1961) The organization of the flight muscle in a dragonfly, Aeshna sp (Odonata). J. Biophys. Biochem. Cytol. 11, 119-145.
54. Stegwee, D. and Van Kammen-Wertheim, A. P. (1962) Respiratory chain metabolism in the colorado potato beetle. - I. Respiration and oxidative phosphorylation in sarcosomes from active beetles. J. Ins. Physiol., 8, 117-126.
55. Tiegs, O. W. (1955) The flight muscles of insects-their anatomy and histology; with some observations on the structure of striated muscle in general. Phil. Trans. B, 238, 221-359.
56. Van den Bergh, S. G. and Slater, E. C. (1962) The respiratory activity and permeability of housefly sarcosomes. Biochem. J. 82, 362-371.
57. Watanabe, M. I. and Williams, C. M. (1951) Mitochondria in the flight muscles of insects. I. Chemical composition and enzymatic content. J. Gen. Physiol., 34, 675-689.

58. Winteringham, F. P. W. (1958) Comparative aspects of insect biochemistry with particular reference to insecticidal action. 4th Intern. Cong. Biochem. (Symposium XII), Vienna.
59. Winteringham, F. P. W. (1959) Presence and significance of α -glycerophosphate in insect tissue. Biochem. J., 71, 21P.
60. Wojtczak, L. and Wojtczak, A. B. (1960) Uncoupling of oxidative phosphorylation and inhibition of ATP-Pi exchange by a substance from insect mitochondria. Biochim. Biophys. Acta, 39, 277-286.
61. Wojtczak, L. and Lehninger, A. L. (1961) Formation and disappearance of an endogenous uncoupling factor during swelling and contraction of mitochondria. Biochim. Biophys. Acta, 51, 442-456.
62. Zebe, E., Delbruck, A., and Bucher, T. (1959) Über den Glycerin-1-P-Cyclus im Flugmuskel von Locusta migratoria. Biochem. Z., 331, 254-272.

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Antonio A. Guerra.

**A COMPARATIVE STUDY OF THE STRUCTURE AND
BIOCHEMICAL ACTIVITY OF FLIGHT MUSCLES FROM SEVERAL
INSECT SPECIES**

**Dissertation submitted to the Graduate Faculty of the
Virginia Polytechnic Institute
in candidacy for the degree of
Doctor of Philosophy
of
Entomology
by
Antonio Alvarez Guerra**

**February, 1965
Blacksburg, Virginia**

ABSTRACT

Comparative histological and biochemical studies were conducted with the flight muscles and mitochondria of several insect species to determine any correlation which exists between muscle structure and the metabolic activity of their mitochondria. Light microscopic examinations were used in the subcellular observations of these muscles. These included direct observations of freshly isolated material (wet mounts), as well as permanent preparations of transverse sections of some muscle fibers. Histological results were presented in a table which includes the muscle type, as well as the fiber, fibril, and mitochondria sizes of several insect species. The presence and abundance of mitochondria and their arrangement in straight rows between the fibrils, was shown to be common in the flight muscles of all insects investigated (except in the female cockroach, Periplaneta americana). In support of these results, a series of microphotographs showing a comparison of the features studied is presented.

Respiratory activity, oxidative phosphorylation, and respiratory control in isolated sarcosomes, were determined in a conventional Warburg respirometer. The importance of using standard isolation and preparation techniques and a standard incubation media was greatly emphasized

in this study. A correlation was found to exist between the sarcosome size and respiratory activity. The smaller the size of the mitochondria the higher its respiratory activity and vice versa. The biochemical similarities exhibited by sarcosomes isolated from all the test species were discussed, and the possibility that these sarcosomes oxidize different substrates with a more or less fixed pattern of relative intensity was suggested.

The relative importance of permeability barriers in the respiratory activity of intact mitochondria isolated from the flight muscles of Periplaneta americana and Musca autumnalis is reported and discussed for the first time in these two species.

The cofactor requirement, their specificity, and their effect on the oxidative capacity of sarcosomes isolated from Periplaneta and Apis thoracic muscles are discussed.

The lack of pigmentation in the white flight muscles of the female cockroach, Periplaneta americana, was investigated, and photographic evidence is given to support the idea that this characteristic is due to the greatly reduced sarcosomal content in these tissues.

The term "pseudo-lamellar" is proposed by the author to characterize the fiber type in the flight muscles of the male cockroach, Periplaneta americana.

These fibers are different than the typical tubular-lamellar muscle fibers of Odonata in that their contractile organelles (fibrils) are small, roughly cylindrical or oval in transversal view, independent, and secondarily arranged in a lamellae-like distribution.

Photographic evidence of the subcellular differences between these fibers, is also given.