CHAPTER 8

A HISTOCHEMICAL STUDY OF THE SLOW AND FAST CONTRACTING LEG MUSCLES OF THE COCKROACH, PERIPLANETA AMERICANA

Some information on certain physiological and histological aspects of the slow and fast contracting muscle fibres of the leg muscles of the cockroach are available from the works of Betch and Dresdon (1956) and Smit (1958). The slow contracting muscle fibres are smaller in diameter and contain more fat than the fast contracting muscle fibres. It is also known that the former possess a stronger capacity to reduce methylene blue than the latter (Smit, 1958). The occurrence of such distinctly different types of muscles situated side by side is not recorded among the leg muscles of other insects. The slow muscle, the tergo-trochanteral muscle is pale red in colour while the fast muscle is white in colour. This contrast in colour is more conspicuous in the muscles of the male than those in the female. The terms 'red' and 'White' muscles are commonly used for muscles whose fibres differ in colour as for example the red and white muscles of fish or rabbit (Ogata, 1960) red and white or broad and narrow fibres in the breast muscle of the pigeon and bat (George et al, 1955, 1958) and the red and white muscles in insects (Edwards and Ruska, 1955). In this connection the slow and fast contracting leg muscles of the cockroach could be called red and white muscles respectively. The cockroach being more of a runner than a flier, the leg muscles are equally well developed as the thoracic

muscles and it is known (Chapter 6) that the thoracic muscles as well as the leg muscles contain a considerably large amount of the enzyme lipase (Chapters 7) as well as oxidative enzymes (Barron and Tanhmisian, 1948). The latter authors also showed that under identical conditions the cockroach muscle is as active metabolically as the pigeon breast muscle. In the light of these findings it was thought desirable to investigate the localization of some enzymes and the distribution of the fuel reserves histochemically in these two types of muscles.

Material and Methods

The cockroaches used in this study were locally collected and kept in the laboratory under uniform conditions. Since female cockroaches are known to be metabolically less active, only male cockroaches were used. To obtain better results the most developed coxal part of the metathoracic leg was used. Due to the small size of the muscle and to avoid injury to the muscles while separating them, fresh frozen sections of the whole coxa were cut according to the method of George and Scaria (1958a) and used for the enzyme work.

Lipase:

The "Tween method" of Gomori using "Tween 80" as the substrate was employed for the histochemical study of lipase. Fresh frozen sections cut as described above were mounted on clean dry slides and allowed to dry for 15 minutes in vacuum

over calcium chloride to ensure adhesion and afterwards fixed in 6% cold (4°C.) neutral formalin for 3 to 4 hours. After fixing, the sections were washed in tap water for 30 minutes and afterwards rinsed thoroughly in distilled water before transferring them to the incubation medium prepared as described in chapter 1. Sections boiled for 10 minutes in water to inactivate the enzyme prior to incubation were taken as control. The incubation period of 6 to 8 hours was found to be the optimum to give good results. Further procedure was as mentioned in Chapter 1.

Alkaline phosphatase:

The histochemical study of alkaline phosphatase was carried out adopting the revised method of Gomori, sodium glycerophosphate being used as the substrate at pH 9.2 . Fresh frozen sections mounted on clean dry slides without any adhesive were dried in vacuum for 15 minutes and fixed in 10% neutral formalin at 4°C. for 1 hour. After fixing, the sections were washed in running water for the same period, rinsed in distilled water and kept in the incubation medium for 24 hours at 37°C. Sections kept in boiling water for 10 minutes before the incubation and incubated along with the samples were used as control. Processing of the incubated sections through cobalt nitrate and yellow ammonium sulphide was done as described in chapter 1. The sections were not counter stained.

Acid phosphatase:

Gomori's revised method (Glick, 1949) using sodium

glycerophosphate at pH 5.1 as substrate was employed to demonstrate the acid phosphatase activity in the fresh frozen sections of the red and white muscles of the cockroach leg. The incubation mixture prepared as described by Glick (1949) was diluted in 1:2 ratio with distilled water and filtered before incubation. The sections were incubated for 6 hours at 37°C. Inactivated sections by keeping them in boiling water for 10 minutes were the controls. After the incubation, the sections were dipped in 2% acetic acid and washed well with distilled water before treating with dilute yellow ammonium sulphide solution. Sections were not counterstained.

ATPase:

Pearse and Reis method (Pearse, 1954) was used to demonstrate ATPase activity in the two types of muscles, using adenosine triphosphate (ATP) as substrate at pH 7.4 . Sections were incubated for 3 hours. The following 3 controls were used. Sections kept in alkaline phosphatase medium at pH 9.2 and 7.4 and sections boiled for 10 minutes before incubation.

Succinic dehydrogenase:

Histochemical localization of succinic dehydrogenase was demonstrated using neotetrazolium as the electron acceptor. The incubation mixture was prepared as described by George and Talesara (1961). The conventional way of incubating sections without mounting on the slides was not suitable for this tissue.

Hence the frozen sections were placed on dry clean slides and dried quickly in a vacuum in 5 minutes to get the sections adhered on the slides. The sections were then incubated as described in an earlier chapter. After in incubation period of 3 minutes the sections were washed well in phosphate buffer and fixed in 10% cold neutral formalin for 1 hour. The sections were mounted in glycerine jelly after washing away the formalin. Sections similarly treated in substrate blank medium were taken as control.

Cytochrome oxidase:

The classical G-Nadi reaction for cytochrome oxidase (Pearse, 1954) was employed on the fresh frozen sections as prepared for the demonstration of succinc dehydrogenase.

Glycogen:

Periodic acid Schiff technique was adopted for staining glycogen, Paraffin sections coated with celloidin were used. Sections incubated in saliva for 30 minutes and stained along with the samples were taken as control.

Results

Lipase:

The dark brown precipitate was found to be more in the fibres of the slow muscle than that in the fast muscle. It was also observed that the precipitate in the slow muscle was comparatively larger and more in number than that of the fast muscle (Fig.1). There were muscle faciculi having precipitates of intermediate sizes.

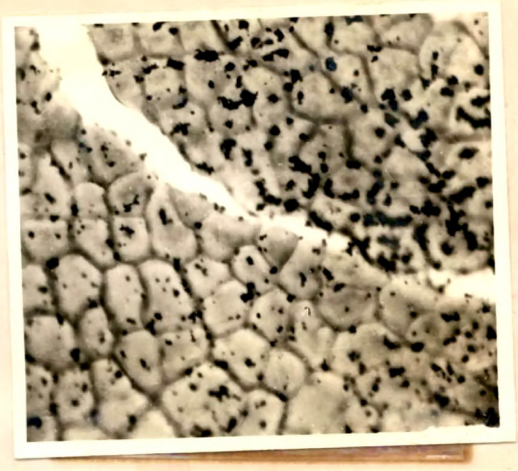


Fig. 1

100 μ

Fig. 1 - Photomicrograph of the slow and fast contracting leg muscles of the cockroach, treated for the localization of lipase.

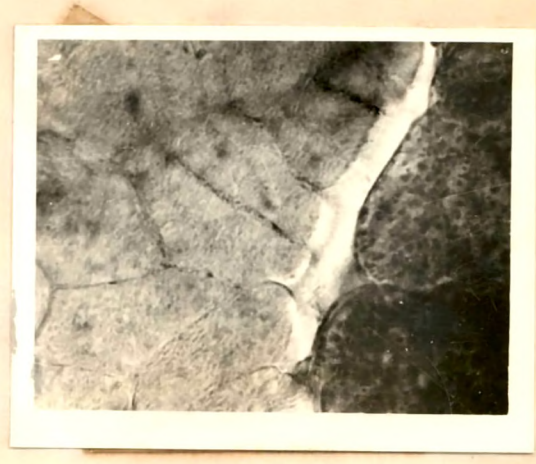


Fig. 2

50 μ

Fig. 2 - Photomicrograph of the slow and fast contracting leg muscles of the cockroach, treated for acid phosphatase.

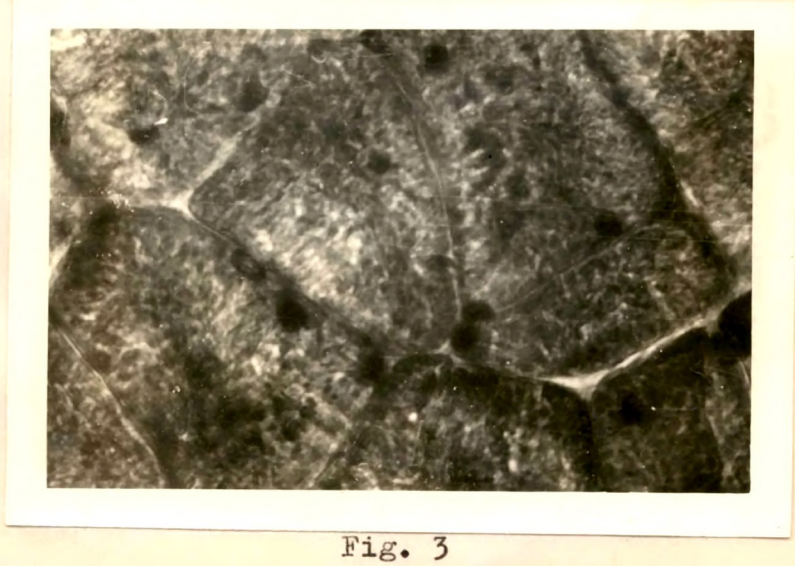
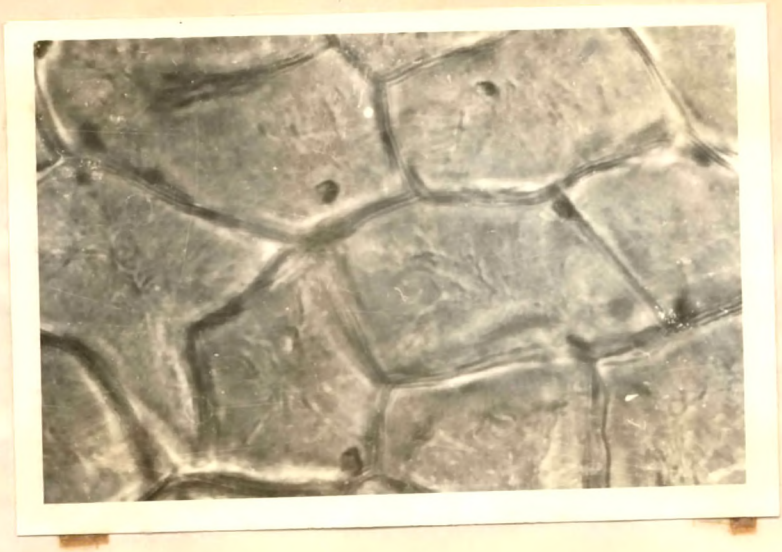


Fig. 3

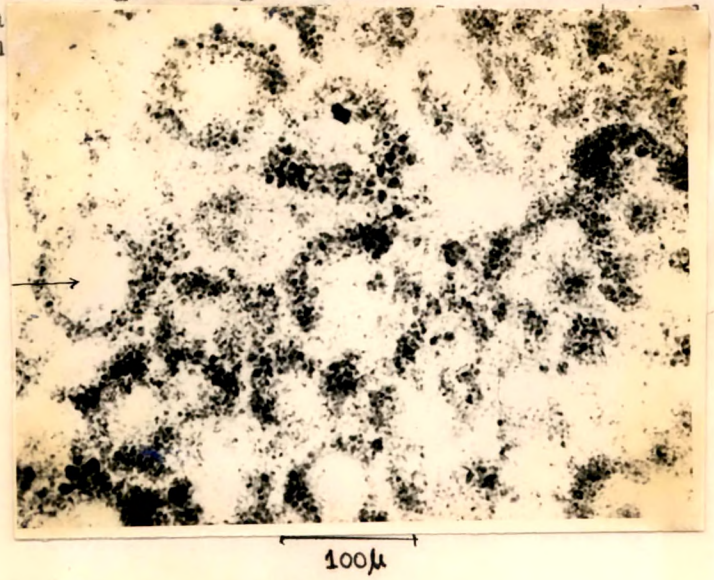
Fig. 3 - Higher magnification of slow contracting muscle fibres from Fig. 2. Note the high activity of acid phosphatase in the nuclei as well as in the sarcoplasm.



50μ

Fig. 4

Fig. 4 - Higher magnification of fast muscle fibres treated for a [illegible] but the sarcoplasm



100μ

Fig. 5

Fig. 5 - Photomicrograph of the slow and fast contracting leg muscles of the cockroach, treated histochemically to demonstrate the disposition of succinic dehydrogenase. The diformazan granules are more in number in the slow muscle fibres than those in the fast muscle fibres.

Alkaline phosphatase:

The intensity of the dark brown colour developed due to the activity of alkaline phosphatase was found more in the red slow muscle than in the white fast muscle. Muscles with intermediate colouration also was observed.

Acid phosphatse:

The activity of this enzyme was also found to be more in the slow muscle than in the fast one. The nuclei in both cases were intensely stained while the sarcoplasmic part of the slow muscle was more coloured than the almost colourless fast muscle (Figs. 2, 3 and 4). Faciculi with intermediate staining also were observed.

ATPase:

As in the case of the other two phosphatases, ATPase activity also was found to be more in the slow red muscle than that in the fast white muscle.

Succinic dehydrogenase:

The concentration of SDH was considerably more in the red slow muscle than that in the white fast one. The slow muscle showed a large number of deep blue diformazan granules whereas the white fast muscle possessed only comparatively less number of these granules (Fig.5). The intermediate type of muscle showed a more conspicuous activity in the case of SDH with more granules than in the fast muscles.

Cytochrome oxidase:

Cytochrome oxidase also showed the same pattern of distribution of the granules as was obtained in the case of succinic dehydrogenase. The activity of this enzyme also was more in the red slow muscle.

Glycogen:

The fibres of the red slow muscle got stained very deeply for glycogen while the white fast muscle fibres gave only a very feeble staining. Intermediate types were found for glycogen staining also.

Discussion

The results of the histochemical observations indicate that the red slow muscle of the cockroach leg is equipped with a better enzyme compliment and the related substrates than the white muscle. It has already been observed (Smit, 1958) that the slow muscle fibres have a higher fat content and a higher metabolic rate than the fast muscle fibres. The structural and certain physiological diffe-rences observed by Betch and Dresdon(1956) and Smit (1958) can well be correlated with the functional activity of these muscles. The results of the quantitative study of the activity of lipase and succinic dehydrogenase in these muscles (Chapter 7) revealed that the slow muscle is metabolically more active than the fast contracting muscles and also the slow muscles possess more potential capacity to utilize more fat for energy during activity.

The biochemical and physiological differences between the red and white muscle fibres of the vertebrates have been investigated by several workers and it was revealed that the red muscle fibres contracted slowly and tonically while the white muscle fibres contracted rapidly and strongly. It is also know that the red muscle fibres are usually smaller in size, contain more mitochondria and more respiratory enzymes than the white muscle fibres which are comparatively larger in size, Since the slow red muscles are continususly contracting, their physiological features may be closely related to their higher activity of oxidation. This seems to be true in the case of the slow contracting leg muscle of the cockroach which showed higher activity for succinic dehydrogenase and cytochrome oxidase. Mitochondria being know as the store houses of the oxidizing enzymes the granules stained in the muscle fibres when treated for these enzymes may be the mitochondria present in them. And the concentration of oxidizing enzymes in any tissue depends upon the mitochondrial content of the same. It is evident from the above contention that the slow contracting red muscles in the cockroach leg possess more mitochondria than the white fast muscle.

The occurrence of alkaline phosphatase in the flight muscles of birds and bat (George, Nair and Scaria, 1958; George, Susheela and Scaria, 1958b) have been histochemically demonstrated and found that the narrow red fibres contain more of this enzyme than the broad white fibres. The red fibrillar

muscle of the beetle stained more for this enzyme than the leg muscle of the same. In the case of the cockroach leg muscles, the slow contracting red muscle stained more darkly for alkaline phosphatase than the fast white musele fibres.

The higher concentration of acid phosphatase and ATPase in the slow contracting red muscle fibres is of special interest. The evidences so far available on the presence of these enzymes show that these two enzymes are found more concentrated in the broad white fibres of the muscles where the fuel resource is mostly glycogen (George and Pishawiker, 1959, 1961). They have shown that acid phosphatase is found in larger quantity in the broad white fibres of the pigeon breast muscle. Similarly they have also shown that ATPase (actomyosin ATPase because mitochondrial ATPase because mitochondrial ATPase, activated by Mg.ions is more in the narrow fibres where the density of the mitochondrial content is very high) is more in the broad fibres of the pigeon (George and Pishawikar, 1959). In lower vertebrates such as fishes and reptiles, the red muscle contains more ATPase, more phosphates and more glycogen (Hotta, 1954, Rodrigues 1959) than those in the white muscle. In the case of the beetle muscles the fibrillar flight muscles stained more intensely for these enzymes than the non-fibrillar leg muscles (Chapter 3).

From the experimental observations, it was found that glycogen stains positively and more deeply in the slow

contracting leg muscle than that in the fast contracting white muscle. In vertebrates the formation of lactic acid is more rapid in the excised white muscle than in the red muscle (Needham, 1926). If this indicates a difference in the rate of distruction of glycogen then the lighter staining for glycogen in the fast muscle of the cockroach could be interpreted as due to its more rapid disappearance during the preparation of the tissue. Quantitative estimation of glycogen in the lower vertebrates have been done and the results obtained show that the red muscle contains more glycogen than the white muscle (Hotta, 1954; Rodrigues 1959).

In all the histochemical observations, apart from the two distinct types of muscles there were present faciculi which gave an appearance of the intermediate type, in colouration and size of the muscle fibres. The recording of the myogram of these muscles by Becht and Dresdon (1956) revealed that the frequency of contractions was of intermediate type, between that of the fast and that of the slow type muscles. The myograms obtained after injecting ryanodine showed that the fast muscles were affected rapidly whereas the slow muscles were affected slowly. The intermediate type of muscles lost its fast element and behaved more or less as the slow muscle. These facts and the observation of the present histochemical study of the slow and fast contracting leg muscles of the cockroach suggest there exist muscles which show features which are combinations of the slow and fast types of muscles. However, further evidence is required to establish the exact catagory of these muscles.