METABOLIC DIFFERENCES AMONG SEVERAL SPECIALIZED INSECT MUSCLES

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INTRODUCTION

It has been known for years that the muscles of insects differ from those of vertebrates and that they also differ considerably among themselves in structure, if not in function. WIGGLESWORTH (1950) has divided the insect muscles into (a) skeletal (elongate and parallel) and (b) visceral (lattices of longitudinal and circular fibers around hollow tubes). All fibers in the insect are striated. These can be divided further into four types according to their histology. These types are: (1) honey bee larva type with centrally located fibrils, large amount of sarcoplasm and peripheral nuclei, (2) common type with little sarcoplasm, evenly distributed fibrils, heavy sarcolemma, and scattered nuclei, (3) "tubular muscles" with fibrils in radial bundles, central nuclei, and little sarcoplasm, and (4) "fibrillar muscles" of the indirect flight muscles of higher insects, consisting os practically isolated bondles of fibrils, weak sarcolemma, heavy tracheation, with nuclei and sarcosomes between and parallel to the fibrils. The first three types are white in color, the fourth type red or brownish.

In a recent survey of the fine structure of these various types of muscles (EDWARDS, SOUZA SANTOS, SOUZA SANTOS, and SAWAYA, 1953; 1954a; 1954b) it has been found that the insect muscles may be divided into two general groups, the "red" and the "white" types. In brief, it was found that the white type (which includes all the muscles of the lower insects, and all the non-flight muscles of higher insects) have a long period, thick sarcolemma, little tracheation, few mitochondria or other inclusions. In isolated preparations the fibrils are flattened (due to high water content), lack N and M lines, and are not uniform in diameter. The red fibers (flight muscles of higher insects) are heavily tracheated, have small period, a weak sarcolemma, large diameter, and are filled with mitochondria. The red fibrils are of uniform diameter, all lines and regions visibly present. In thin sections (EDWARDS and RUSKA, 1954) the white fiber was found to have a thick sarcolemma, few intracellular tracheoles, few mitochondria, and is characterized by a synfibrillar system enclosing an endoplasmic reticulum. The red fiber was characterized by a thin sarcolemma enclosing an extensive intracellular tracheolar-mitochondrial system surrounding widely spaced, individualized fibrils.

It is to be noticed that in the lower insects all the muscles are of a

single color within a given individual, varying from translucent white to yellowish or pinkish. The color may vary very slightly from individual to individual, or may vary with sex and age. In the higher insects the amount of red muscle increases with advancing evolutionary position, i. e. in *Belostoma* the flight muscles occupy a small portion of the thorax; the major portion being filled with the large, white, extrinsic, leg muscles. In the bee or fly, however, the thorax is practically entirely filled with red, flight muscles; the white muscle consisting of a few small, extrinsic, leg muscles found near the coxa.

Among the various muscles of insects there may be found varying physiological properties, e. g. absolute power, duration of twitch, latency, chronaxie, etc. (cf. WIGGLESWOTH, 1950). More to the point of the present paper, however, are those differences found during flight. In a survey of five insects, ROEDER (1951) found that they could be divided into two groups according to their neuro-muscular responses during glight. The first group, *Periplaneta* and *Agrotis,* have a wing rate less than 40 per second, with a conventional neuro-muscular excitation, have a ratio of 1-1 between wing beat and spike potential, and show little or no second type, represented by *Vespa, Calliphora,* and *Lucilia,* have a wing beat greater than 100 per second, a ratio between wing beat and spike change in frequency of thoracic vibration following wing amputation. The potential of 5 to 16, and show a marked increase in wing rate, and hence w/s, following amputation. Thus ROEDER concluded that the higher forms have developed specialized motor mechanisms in the flight muscle which differ considerably from that of the more conventional neuro-muscular mechanism of the lower forms; this mechanism being in the direction of a myogenic system.

The biochemistry of insect muscle is ably reviewed by GILMOUR (1951) and will not be attempted here. In general, the studies have shown that the intermediary pathway in the insect is similar to that of the vertebrate. Certain studies, however, are quite to the point of the present paper, hence shall be mentioned here in somewhat more detail. As early as 1925, KEILIN, in his survey of the presence of cytochrome in the animal kingdom, found that the highest concentration of this substance, among all organisms, was in the flying insect thoracic muscle. Other insect muscles showed little cytochrome, and indeed, in the muscles of the non-flying, or wingless insects, such as the sheep ked, the presence of cytochrome was hardly detectable. It is notable that the metabolic activity of all insect muscle appears to be higher than that of vertebrate muscle. Cockroach muscle, for example, shows an oxyigen consumption and activity of cytochrome c, DPN and DPT inferior only to that of the most active vertebrate muscle, the pigeon breast muscle (BARRON and TAMISLAN, 1948). The diferences among the various muscles of the insects appear to be related to the number of sarcosomes, the mitochondria of insect muscle. These bodies have

most of the activity shown for mammalian mitochondria, principally that of the oxidases and certain dehydrogenases, and have a similar chemical composition (WATANABE and WILLIAMS, 1951). They also resemble vertebrate mitochandria in theiv morphological changes and permeabilite, characteristics (WATANABE and WILLIAMS, The cyitochromes appear to be located within the mitochondria, rather than in the fibrils (WATANABE and WILLIAMS, 1951) the concentration varying with age and activity. SACKTOR (1953) has shown that muscles of higher color (due to greater concentrations of mitochondria) have more cytochrome c and cytochrone c reductase activity. The mitochondria of the flight muscles of the house fly contain a specific ATPASE, activated by MG and MN, which splits the terminal phosphate of ATP, whereas the ATPASE of the fibrils can utilize other phosphates and is CA activated (SACKTOR, 1953). The house fly mitochondria can also oxidatively synthesize high energy phosphate bonds (SACKTOR, 1954). The fibrillar ATPASE thus resembles that of the lower insects (SACKTOR, THOMAS, MOSER, and BLOCH, 1953; GILMOUR and CALABY, 1953a; 1953b). The cockroach ATPASE is inhibited by p-chloromercuribenzoate but not by azide and fluoride. The ATPASE of house fly mitochondria, however, is inhibited by azide as well the benzoate (SACKTOR, 1953).

With the evidence thus pointing to a difference between lower and higher insect muscle, particulary with regard to structural and physiological differences between red and white muscle, we have thus commenced a study of the metabolism of flight and leg muscles from various insects. To date information has been obtained on the oxygen consumption and dehydrogenase activity. The results fit in with the above evidence to give a rather clear picture of the evolution of a special type of muscle for the accomplishment of the rapid movements of flight in the higher insects.

MATERIAL AND METHODS

The insects used in the present experiments were adults of the following species: *Periplaneta americana, Hydrophilus ater, Schistocerca infumata,* and *Belostoma* spp. The aquatic insects were obtained from a small pond on the outskirts of São Paulo and created within the laboratory in aquaria; simulating natural conditions as much as possible. *Schistocerca* was obtained in fields at the city limits and the *Periplaneta* came from the general laboratory supply. The muscles used were generally the dorso-longitudinal, indirect, flight muscles and the coxal levators. In some cases other flight muscles, as well as femoral muscles, were used.

The oxygen consumption was determined in volumetric micro-respirometers (SCHOLANDER, 1942) adapted for tissue study. The procedure was as follows. The insect was opend, moistened with saline, the

muscle rapidly dissected out, blotted with filter paper and weighed fresh. Ten to 20 mgms. of muscle were then removed rapidly to the experimental vessel of the respirometer which contained sufficient saline to give a final suspension equivalent to 25 mgm. muscle per 0.1 cc. saline. The saline thus covered about one half of the muscle surface. The muscles were then gently teased apart by needles until a suitable surface area was exposed. The experiments were run at 25°C for a minimum of 1 hour; generally being from 1 to 2 hours in duration. Dry weight was not always taken in individual experiments. It was usually determined by weighing large quantities of muscle fresh, then drying at 100°C for 24 hours and weighing dry, thus by using larger quantities of muscle the errors of weighing in individual lots could be lessened.

The dehydrogenase activity was determined by the FRIEDEMANN and HOLLANDER (1942) modification of the Thunberg technique, using reduction of methylene blue as the criterion. The muscle was used in a suspension of 5% by weight in insect saline. Experiments were run at 25°C. In the control experiments saline alone was used. For total endogenous dehydrogenase activity no substrate was added. For succinic dehydrogenase activity, succinate at 0.5 molar, was used to replace part of the saline.

Two insect salines were used in the experiments. Initially we used that of YEAGER, which in its composition is close to insect hemolymph, but inasmuch as the results obtained were erratic and lower than expected we changed to the use of the saline of WILDER and SMITH. In agreement with our experience the latter saline has been shown to be the best saline for insect heart action (MENDES, 1954).

RESULTS

1. *Distribution and color of muscles*:

The muscles used in the present study were chosen on the basis of their specific function and color, inasmuch as electron microscope studies had previously demonstrated structural differences related to these factors. In *Periplaneta americana* the various muscles of the body are of a single color within a given individual. The color is, in general, amberish-white, but may vary from translucent white to pinkish-yellow. The muscles of the male are usually darker than those of the female, and in general the muscles become darker with increasing age in both sexes. Slight individual differences may occur whether the individuals be of the same sex or age. Despite these slight variations it can be stated that *Periplaneta* has a single type of muscle, i. e. the "white" type. *Schistocerca* shows a similar distribution and type of musculature but differs slightly in the coloration. In general the body musculature is of the same color as the dark *Periplaneta* muscle; being a yello-

wish-pinkish-brown, thus approaching the color of the wing muscles of the higher insects. The structure, however, is that of typical white muscle.

Hydrophilus and *Belostoma* are quite representative of the higher insects in their muscle coloration in relation to structure and function. In both insects the indirect flight muscles are a pinkish brown, whereas the other muscles of the body are a translucent white. The indirect, glight muscles are heavily tracheated and filled with mitochondria, in contrast to the white muscles which are poorly tracheated and show few mitochondria. The difference in coloration of the two types of muscles appears quite clearly and is uniform i. e. little variation from one individual or sex to the next. The color of the red muscle is mainly due to the mitochondria. By differential centrifugation and filtration it is possible to obtain two fractions, one of which is chiefly mitochondria with few fibrils and the other chiefly fibrils. However, both fractions have color, the first being quite reddish, the second a lighter pink. Microscopic examination shows that the mitochondria fraction may be relatively pure, but the fibril fraction always contains a large number of mitochondria adhering to the fibrils. Thus we must consider that part of the color of the red muscle may be due to the fibrils. This is certainly the case in the dark muscles of *Schistocerca* where microscopic examination shows few mitochondria.

2. *Oxygen consumption*:

The oxygen consumption of flight and coxal (*Schistocerca*, femoral muscle) was measured in the four insects. Before discussing the definitive data two points of interest in experimentation should be mentioned.

Firstly, it was found that the type of saline used had a considerable influence on the results obtained. YEAGER'S saline, close in its composition to that of insect hemolymph, and containing magnesium, gave more erratic and lower oxygen consumption values than did the saline of WILDER and SMITH. For example, the oxygen consumption of flight muscle of *Hydrophilus* in YEAGERùS saline averaged 0.551 mm3 $^{o}2$ mgm hr. whereas in the saline of WILDER and SMITH in averaged 1.91. A similar difference existed for coxal muscles, giving values of 0.091 in YEAGER'S saline and 0.416 in the saline of WILDER and SMITH. That this discregancy is not due to the particular insect is shown by the fact that similar results were obtained with *Belostoma*, in which values for the two types of muscles in the respective salines were: flight muscle — YEAGER, 0.412; WILDER and SMITH, 1.16; and coxal muscle — YEAGER, 0.147; WILDER and SMLTH, 0.308. The difference btween the two salines appears to be due to the presence of magnesium and the ratio existing between NA, K, and CA (cf. MENDES, 1954).

Secondly it was found that the amount of tissue used may influence the results. Stirring is not used in the SCHOLANDER micro-respirometer due to the fact that the amount of tissue is small and diffusion appears not to be limiting. In the present experiments a limit appeared to be reached with 30 mgms. of tissue. The usual technique was to place the entire muscle in the flask and then tease the fibers gently apart with needles in order to provide maximum surface area for oxygen consumption. It was found that with pieces of muscles under 30 mgms. teasing was not necessary, the results being the same, for let us say, a 20 mgm. muscle with or without teasing. For pieces of muscle over 30 mgms. In weight the oxygen consumption was lower per unit weight, hence presumably diffusion was poor in these cases. To avoid any difficulty pieces of muscle of 10 to 20 mgms. were used and teased in the definitive experiments. It was noted also that the saline should be used in a quantily that would cover no more than one half the muscle surface.

The results of the definitive experiments are presented in Table One. As may be seen, the rates of oxygen consumption of the flight and leg muscles of the two lower insects, *Periplaneta* and *Schistocerca*, were essentially similar. The average QO_2 in the *Periplaneta* male was 1.72 for flight muscle and 1.50 for coxal muscle, and in the female 1.21 and 1.04 respectively, giving ratios of F/C of 1.1 and 1.2. Thus, as might be expected, the oxygen consumption of the muscle of the male was greater than that from the female, but the oxygen consumption of the flight muscle, in both sexes, was only very slightly greater than that of leg muscle. *Schistocerca* flight muscle exhibited a rate of oxygen consumption slightly arger than that of the leg (femur) muscle, with values of 1.67 and 1.22 respectively, giving a ratio of 1.3. In comparison with the figures to be presented below for the higher insects it will be seen that this difference between *Schistocerca* flight and leg muscle is practically insignificant. It is to be noted that the absolute values of oxygen consumption for the six muscles above (average 1.40) are higher than those usually encountered in vertebrate muscle. On a dry weight basis the rate of oxygen consumption becomes about five times higher, varying slightly from muscle to muscle due to slight variations in water content, but the ratios do not change significantly.

In the two higher insects, the red, flight muscle showed a higher oxygen consumption than the white, leg muscle (coxal levator); being in the WILDER and SMITH solution 1.91 as compared with 0.416 in *Hydrophilus*, and 1.16 in contrast to 0.308 in *Belostoma*, giving ratios of 4.6 and 3.8 respectively on a fresh weight basis. The same relationship obtains in YEAGER'S solution, but with different absolute values, i. e. in *Hydrophilus* 0.551 and 0. 091, and in *Belostoma* 0.412 and 0.147, giving ratios of 6.1 and 2.8 respectively on a fresh weight basis. On a dry weight basis, due to considerable differences in water content of

TABLE ONE

OXYGEN CONSUMPTION OF FLIGHT AND LEG MUSCLES

Insect	mm3 02/mgm./hr.				Ratio		Saline
	flight		leg		flight/leg		
	fresh	dry	fresh	dry	fresh	dry	
Hydrophilus ater	0.54 0.535 0.720 0.22 0.74		0.138 0.095 0.091 0.041				YEAGER
	0.551	1.64	0.091	0.418	6.1	3.9	
	1.50 1.96 2.00 2.00 2.20 1.80		0.56 0.335 0.145 0.134 0.66 0.66				WILDER and SMITH
	1.91	5.71	0.416	1.91	4.6	2.9	
Belostoma spp.	0.50 0.45 0.304 0.402 0.402		0.125 0.131 0.070 0.093 0.315				YEAGER
	0.412	2.02	0.147	0.82	2.8	2.5	
	0.86 1.00 1.63		0.119 0.339 0.465				WILDER and SMITH
	1.16	4.23	0.308	1.43	3.8	2.9	
Schistocerca infumata	1.80 2.25 1.06 1.58		1.56 1.04 1.05				WILDER and SMITH
	1.67		1.22		1.3		
Periplaneta americana male	1.70 2.20 1.75		1.86 1.40 1.83 1.87 1.87				WILDER and SMITH
	1.88	7.30	1.55	6.2	1.1	1.1	
Periplaneta americana female	1.42 1.10 1.10		1.03 1.08 1.01				WILDER and SMITH
	1.21	4.54	1.04	4.41	1.2	1.0	

the several muscles, the ratios change, averaging from 2.46 to 3.93. Thus, in both insects in both salines the flight muscle showed a considerably higher oxygen consumption than the leg muscle. Only in *Hydrophilus* was the flight muscle oxygen consumption higher than that of the muscles of the lower insects; the white, leg muscles of the higher insects having a lower oxygen consumption than the various lower insect muscles.

3. *Dehydrogenase activity*:

The dehydrogenase activity was determined by means of a modification of the THUNBERG technique using as the criterion the time for the reduction of methylene blue under agar. Several different techniques were employed to determine the most applicable method. Muscle tissue in small chunks gave rapid reduction of the dye in the agar immediately surrounding the tissue but gave poor and uneven overall reduction. Homogenised tissue gave the most uniform reduction but the time required was so long as to be inconvenient. The best method, giving both uniformity and rapidity of reduction, was to triturate the muscle in saline by hand in a small mortar for several minutes until the pieces were fine enough to passa freely through the capillary of a tuberculin syringe, using a concentration of muscle 5% by weight.

The results (Table Two) confirmed the results obtained in the measurement of oxygen consumption, i. e. little difference between the several muscles of the lower insects, but a large difference between the relationship was found between the reduction times of flight and leg muscles in the female (94 and 90 minutes respectively) and a 1.3 ratio in those of the male (14 and 18 minutes respectively) for total endogenuos dehydrogenase activity. In *Schistocerca* the flight muscle showed 3.4 times the activity of the leg muscle (11 and 37 minutes respectively). Thus the results confirm those of the oxygen consumption experiments. Further, as previously shown (BARRON and TAHMISIAN, 1948) the enzyme activity of the male cockroach muscle is considerably greater than that of the female. The results obtained with *Schistocerca* are less easily explained. On the basis of the relative rates of oxygen consumption one would not expect such a difference in dehydrogenase activity. However, is has been shown previously (GILMOUR and CALABY, 1953b) that the pyrophosphate activity of the flight muscle of the locust is approximately three times that of the femoral muscle.

The addition of succinate decreased the reduction times for the various muscles, but did not alter the ratios.

In the two higher insects the reduction time for the flight muscles was considerably less than that for the leg muscles. In *Hydrophilus* the endogenous activity of the flight muscle was 20 times greater than that of the leg muscle, and the succinic dehydrogenase activity 14 times greater. In *Belostoma* the endogenus dehydrogenase activity of the

TABLE TWO

DEHYDROGENASE ACTIVITY OF FLIGHT AND LEG MUSCLES

Insect	Minutes for reduction of Meth. Blue				Ratio		Saline
	flight		leg		leg/flight		
	endogenous	succinic	endogenous	succinic	endogenous	succinic	
Hydrophilus ater	9	3	170	40			all experiments in WILDER and SMITH saline.
	11	3	150	35			
	7	2	169	36			
	7	3	180	37			
	8	2	180	40			
	8.4	2.7	170	38	20.2	14.1	
Belostoma spp.	10	2	150	22			
	10	2	150	25			
	10	2.5	160	30			
	10	2.2	153	25.7	15.3	11.8	
Schistocerca infumata	10		40				
	10	1.5	20				
	12	2	46				
			40				
	10.7	1.8	36.5		3.4		
Periplaneta americana male	20		20				
	12		18				
	10	2.5	15	4			
	18	3.0	20	3			
	11	2.5	17	3			
	14	2.7	18	3.3	1.3	1.2	
Periplaneta americana Female	90	20	90	20			
	91	20	95	19			
	100	20	84	20			
	94	20	89.7	19.7	1.0	1.0	

flight muscle was 15 times greater, and the succinic dehydrogenase activity 11 times greater than those of the leg muscle.

As in the case of the oxygen consumption, the differences were due more to the lower activity of the leg muscles than to an increased activity of higher insect flight muscle over that of lower insect flight muscle. As may be noted in the table, the endogenous activity of the flight muscles of the four insects was similar (8.4, 10, 14, and 10.7 minutes) and the succinic activity also essentially the same (2.7, 2.2, 2.7, and 1.8 minutes) with the exception of the *Periplaneta* female. The white muscle activity of the two higher insects, however, was distinctly less than that of the leg muscle activity of the two lower insects, including that of the *Periplaneta* female.

DISCUSSION

The results have shown that the oxygen consumption, total endogenous dehydrogenase and succinic dehydrogenase activity of flight and leg muscle of *Periplaneta* are similar. The oxygen consumption of flight muscle of *Schistocerca* is very slightly higher than that of the leg muscle, but the dehydrogenase activity of the former is three times that of the latter. In the two higher insects the flight muscle oxygen consumption is three to four times, and the dehydrogenase activity 15 to 20 times that of the leg muscle. However, the absolute values for the higher insect flight muscles are equivalent to those for the lower insect muscles, thus the difference is acounted for principally by the lower activity of the leg muscle of the two higher insects. Accompanyng these activities we find a change in color of the muscles, i. e. single pale color in all muscles of the lower insects, and a definite division of color in the higher, i. e. red flight and white non-flight muscles. Also notable is the difference in separability of gibers, the red muscle easily separating out into single fibers, the white muscle more difficultly so. By differential centrifugation it is possible to note that the red color of the higher insect flight muscle is due principally to the presence of myriads of large, reddish mitochondria4 By microscopic examination one can note that the white muscles lack these mitochondria but have a reticular system and few small mitochondria between the fibrils.

On the basis of these facts, plus the known morphological and biochemical events in insect muscles, it appears quite evident that there has occurred an evolution of insect muscle from a single, all purpose type into several highly specialized types for such activities as fast flight and strong support. The evidence for this idea can be put together as follows.

Structurally the muscles of the lower insects are all of a single type and this type is similar, in general, to the white muscle of the higher insects. The white muscle is characterized by relatively little tracheolization, few small mitochondria, an endoplasmic reticulum, and a

synfibrillar arrangement within the fiber, thus giving a structure more adapted to slowness of action and great mechanical strength linked with a low oxygen demand (EDWARDS and RUSKA, 1954). Insects with the single type of muscle, i. e. lower insects, are characterized by a low wing rate, a wing beat — spike potential of 1 and no change in frequency with modification of wing length (ROEDER, 1951). On the other hand, the red, flight muscle of higher insects is characterized morphologically by an intracellular tracheole-mitochondria system surrounding the widely spaced, individual fibrils, thus being a structure adapted to rapidity and flexibility of action, with high oxygen needs (EDWARDS and RUSKA, 1954). The higher insects, indeed, demonstrate this in that they possess a high velocity of wing beat, a high wing beat — spike potential ratio, and a change in beat with modification of wing length, thus tending in the direction of myogenicity in the wing muscles (ROEDER, 1951).

Allowing these facts one still has to explain what has happened within the fiber that would cause no change in the flight muscle metabolic requirements and cause a lowering of the white muscle requirements. The logical answer appears to be two fold. Firstly, the arangement of elements within the fiber i. e. the hig hfibril — sarcoplasmic ratio and synfibrillar arrangement within the white muscle argue for mechanical efficiency and strength, whereas the widely spaced fibrils and the tracheole-mitochondrial continuum surrounding the fibrils, thus placing the necessary energy over a tremendous surface area, argue for metabolic efficiency for rapidity of mechanical action. Secondly, and logically, there must have been a shift of site of oxidations and phosphorylations to accompany the morphological changes, i. e. a shift from the fibril in the white fiber to the mitochondria in the red. Unfortunately we do not have sufficient comparative information to state this as more than a working hypothesis, but certain recent biochemical studies certainly point in this direction.

If we start on the premise that lower insect muscle and higher insect white muscle is essentially a fibrillar mass, and that the red, flight muscle is essentially a mitochondrial mass, then the information concerning the lower insect muscle is essentially information about fibrils and can be used in conjunction with our information of the fibrils of higher insets, giving us thus the opportunity to compare fibrillar and mitochondrial activity, as follows.

The cockroach leg muscle has a cytochrome c content higher than that of pigeon breast muscle, has a DPN content twice that of rat muscle, contains a transaminase, is the site of the succinoxidase system and utilizes the KREBS-SZENT-GYORGYI cycle (BARRON and TAHMISIAN, 1948). On the other hand, in the Diptera the cytochromes and the succinoxidase system are located exclusively in the mitochondria, and the mitochondria are capable of utilizing the intermediates of the KREBS-SZENT-

GYORGYI cycle (WATANABE and WILLIAMS, 1953; 1954; SACKTOR, 1953; 1954).

The fibrils of the house fly flight muscle have a CA activated ATPASE whereas the mitochondria have a MG and MN activated ATPASE (SACKTOR, 1953).

As intermediate form is perhaps to be found in the locust. The morphology of the locust flight muscle appears to be intermediate between that of the cockroach and the "fibrillar' type of higher insects (GILMOUR and CALABY, 1953a), the pyrophosphate activity of its flight muscle is about 3 times that of the femoral muscle and the MG apyrase activity is greater in the flight than in the leg muscle (GILMOUR and CALABY, 1953a; 1953b). In addition, locust muscle myokinase converts ADP to ATP and AMP (GILMOUR and CALABY, 1953b) which can be considered comparable to the ability of the house fly mitochondria to oxidatively phosphorylate ADP to ATP (SACKTOR, 1954).

Thus, coinciding with the structural changes in the muscles, we find a metabolic shift, in that the activity associated with the fibrils in the lower forms is associated principally with the mitochondria in the higher forms.

One further point remains to be considered, that is, the comparison of the insect muscle with the vertebrate muscle in these two respects, morphology and metabolic activity. The insect white muscle appears to be close in structure and quantity of activity to the vertebrate muscle, i. e. structurally with its few mitochondria and its endoplasmic reticulum, and functionally in that in the vertebrate muscle the phosphate compounds are located within the I region of the fibril, the ATPASE presumably in the A region, with the endoplasmic reticulum supplying the nucleotides; the sarcoplasm sopplying the glycogen and the 3C compounds being oxidized by the adjacent mitochondria (RUSKA, 1954) The insect red, flight muscle, however, lacks the reticulum, has huge quantities of mitochondria and the enzymatic components appear to be located within these mitochondria. Thus again we have evidence that the insect has made a shift in the direction of a highly efficient metabolically and structurally specialized muscle for fast flight.

SUMMARY

The oxygen consumption, total endogenous dehydrogenase and succinic dehydrogenase activity of flight and leg muscle has been determined in *Periplaneta americana*, *Schistocerca infumata*, *Belostoma* spp., and *Hydrophilus ater*.

The two lower insects have muscles of a single color within a given individual, the color averaging pale amber. The color may vary slightly with sex and age. The two higher insects have muscles of two colors; the flight muscle being reddish and the other muscles of the body being white. The color of the red, flight muscle appears to be due mainly to the mitochondria.

The rates of oxygen consumption of the flight and leg muscles of male *Periplaneta* and *Schistocerca* were of the same order of magnitude. The oxygen consumption of the two muscles from the female *Periplaneta* was the same, but lower than that of the male.

The oxygen consumption of the flight muscle of *Hydrophilus* was 4.6 times that of the leg muscle, and of *Belostoma* was 3.8 times on a fresh weight basis. The oxygen consumption of the flight muscles was similar to that for the two lower insects. The leg muscle oxygen consumption was lower than that of the muscle of the two lower insects.

The ratio of total endogenous dehydrogenase activity, and succinic dehydrogenase activity of the flight muscles and leg muscles of *Periplaneta* was essentially unity. In *Schistocerca* the flight muscle showed 3 times the activity of the leg muscle.

The total endogenous dehydrogenase activity ,and succinic dehydrogenase activity of the flight muscles of the two higher insects was 15 to 20 times that of the leg muscles. The differences were due more to the lower activity of the leg muscles, than to an increase in activity of the wing muscles over that displayed by the lower insect muscle.

A discussion is presented in which these results are integrated into previous findings in support of a theory on the evolution of insect muscle.

SUMÁRIO

Foi feito um estudo comparativo entre músculos do vôo e músculos das pernas do seguintes insetos: *Periplaneta americana, Schistocerca infumata, Belostoma* spp., e *Hydrophilus ater*.

1. *Coloração dos músculos*: — Nos dois insetos inferiores, *Periplaneta* e *Schistocerca,* todos os músculos do corpo, num dado indivíduo, apresentam-se com a mesma coloração, mais ou menos pálida; mas, de um indivíduo para outro a côr pode variar com o sexo ou com a idade. Os dois insetos superiores, *Belostoma* e *Hydrophilus,* têm músculos de duas côres: os do vôo são avermelhados, e todos os outros músculos do corpo são brancos. A côr dos músculos do vôo parece ser devida principalmente às mitocôndrias.

2. *Consumo de oxigênio*: — Tanto em *Schistocerca* como em *Periplaneta* não há uma diferença sensível entre o consumo de oxigênio dos músculos do vôo e dos músculos das pernas. Em *Periplaneta* observa-se uma diferença entre os dois sexos. Os músculos da fêmea que são bem mais claros que os do macho, têm menor taxa de consumo de oxigênio.

Em *Hydrophilus* e *Belostoma,* o consumo de oxigênio dos músculos do vôo é respectivamente 4,6 e 3,8 vêzes maior que o consumo de oxigênio dos músculos das pernas, sendo que a taxa do consumo de oxigênio dos músculos do vôo dêstes dois insetos é da mesma ordem da dos insetos inferiores, enquanto os músculos brancos das pernas mostram muito menor atividade (Tabela 1).

3. *Atividade dehidrogenásica*: — Também na atividade dehidrogenásica, endógena e succínica, em *Periplaneta,* não há diferenças entre músculos do vôo e músculos das pernas, e os músculos do macho são 4 a 5 vêzes mais ativos que os da fêmea. Em *Schistocerca,* os músculos do vôo mostram uma atividade 3 vêzes maior que a dos músculos do femur.

Nos dois insetos superiores, as diferenças encontradas entre os dois tipos de músculos são muito maiores. Os músculos do vôo mostram-se 15 a 20 vêzes mais ativos que os das pernas. Aqui também as diferenças são devidas mais a pequena atividade dos músculos brancos que a um aumento na atividade dos músculos vermelhos, em relação à atividade dos músculos dos insetos inferiores (Tabela 2).

4. *Discussão*: — E' apresentada uma discussão onde os dados do presente trabalho são integrados com resultados prévios de outros autores com a finalidade de demonstrar que: 1. a coloração do músculo evidencia riqueza em enzimas que contém ferro; o que explica o metabolismo mais intenso dos músculos com coloração mais carregada; 2. há uma evolução na musculatura, a partir dos insetos inferiores com um só tipo de músclos para os insetos superiores com músculos especializados para o vôo e outros especializados para sustentação.

Morfològicamente os músculos dos insetos inferiores e os músculos brancos dos insetos superiores podem ser reunidos num só grupo com as seguintes características: fibras musculares com sarcolema espêsso, com arranjo sinfibrillar, o que dificulta a separação das fibrilas, escassa traqueolização, poucas mitocôndrias e um sistema reticular entre as fibrilas. Os músculos vermelhos do vôo caracterizam-se por fibras com sarcolema finíssimo, por fácil separação das fibrilas, pela extraordinária quantidade de mitocôndrias grandes, e traqueólas que circundam as fibrilas espaçadas.

Equipamento enzimático e oxigênio, fàcilmente disponíveis, ao redor das fibrilas, favorece fácil entrega de energia para a contração rápida das fibrilas flexíveis do músculo vermelho.

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