EFFECT OF PHYSICAL STRESS ON THE PRAWN *PENAEUS INDICUS* H. MILNE EDWARDS

DISSERTATION SUBMITTED BY
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COCHIN - 682 031

CERTIFICATE

This is to certify that this Dissertation is a bonafide record of the work done by Kum. Bindu R. Pillai under my supervision and that no part thereof has been presented before for any other degree.

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CONTENTS

P R E F A C E

In intensive and semi-intensive aquaculture practices the animals are under stress conditions. These stress factors can be environmental, biotic and abiotic, toxic or manmade. Environmental changes leading to stressful situation have been shown to alter the animals' respiration and metabolism to a great extent. In addition, procedures adopted in aquaculture techniques such as handling of animals, confinement, transport and disease treatments cause a variable degree of trauma in them. These factors can adversely affect the overall production of animals in a culture system.

Thus stress and stress responses are of immediate economic importance in intensive farming system where the animals are always subjected to many chronic stressors. Therefore, an understanding of the responses of such cultivable animals to acute physical stress is also of paramount importance to field biologists, who often handle such animals prior to or during their investigations.

Acute forms of handling stress almost invariably results in marked physiological stress responses and if severe enough, can have lethal consequences. Chronic stressors have been found to be associated with reduced growth, and

increased susceptibility to pathogens. In addition, the primary stress factors can reduce the animals' ability to withstand a secondary stressors. Thus it is necessary to consider the stress aspects as prime importance in any culture system so as to minimize the effects and if necessary, modify the procedures of handling.

The cultured animals are often confined and captured by a variety of nets, electrical devices, or even slow poisoning. The violent movement of animals in escaping from such adverse situation may result in marked metabolic changes. The energy demand during the stress period often causes an increased metabolic rate that exceeds the animals' ability to consume oxygen. At such times body tissues generally resort to anaerobic production of energy, accumulating metabolic end products which are often highly acidic. Such an acidosis could be the cause of death and post stress reductions in product quality.

Among the cultivable aquatic organisms, penaeid shrimps are commercially very important as they grow faster in confined water bodies, attain larger size and fetch a high price. Among the penaeid prawns, P. indicus is of particular interest not only because of its abundance but also of its hardiness in culture. In India, it forms the major candidate species used in extensive and semi-intensive culture

systems. In recent times the traditional prawn culture practices are widely replaced by semi-intensive and intensive culture systems. Owing to natural and man made manipulations, the aquaculture system is often exposed to variations in the biotic and abiotic factors which inturn affect the culture of species to a greater extent. Quite frequently, prawn farmers face mass mortality of their stock mainly due to the stress of adverse environmental conditions. The most suspected cause of the much talked 'soft prawn' disease commonly encountered in prawn farms may be the t: widely fluctuating environmental conditions. Opaqueness in the abdominal musculature of prawns observed during harvesting time in many of the prawn farms may be due to severe physical stress on the animal during capture and transportation. The opaqueness usually appears as focal areas of necrosis. This condition is reversible in its initial stages, but it may be lethal if large areas are affected. Histopathological and etiological investigations suggest that the possible cause of this condition may be related to severe exertion caused in efforts to escape.

The present investigation on Penaeus indicus H. Milne Edwards, has been conducted to examine the effect of physical stress on the various biochemical constituents such as glucose,

lactic acid, lipid and protein in haemolymph and glycogen, lactic acid, lipid and protein in muscle tissue. Studies were also made on histological changes occurring in hepato-pancreas and muscle during stressed conditions.

I wish to express my deepest sense of gratitude to Dr. P.S.B.R. James, Director of Central Marine Fisheries Research Institute, under whose scholarly guidance this work has been completed. I am grateful to Dr. A.D. Diwan, Scientist S-2, for his valuable advice and help. It is my pleasure to thank Dr. A. Laxminarayana for his help in procuring the specimen and Mr. K.N. Kurup for his help in statistical analysis. My gratitude is also due to Mr. Nandakumar for his timely help. I am deeply indebted to my classmates and seniors, especially Laxmilatha and Sahul Hameed for their valuable advice and help. I express my gratitude to the Indian Council of Agricultural Research for awarding me a Junior Research Fellowship during the tenure of which this study has been carried out.

I N T R O D U C T I O N

The concept of biological stress has evoked much discussion and disagreement in the scientific circle and as a result, there are a variety of formal definitions in the scientific literature. However, all definitions of stress share the common premise of a stimulus acting on a biological system and the subsequent reaction of the system (Pickering, 1980). Professor Hans Selye defined stress as a state of non-specific tension in living matter (Selye, 1946). His studies on mammalian systems showed that the stress occurs in three phases such as alarm, resistance, and exhaustion. The first occurs immediately after the stimulus, in the second a new steady state is maintained at the expense of metabolic energy and finally exhaustion occurs as reserves are used up.

Brett (1958) modified the definition of stress as a state produced by environmental or other factors which extends the adaptive responses of an animal beyond the normal range. In the light of molluscan studies Bayne (1975) further modified the definition of stress and according to him, stress is defined as a measurable alteration of a physiological steady state which is induced by an environmental change, and which renders the individual more vulnerable to further environmental changes.

Aquaculture of captive fish and shellfish can-not be undertaken without the use of various handling procedures in the field, hatchery, or in the laboratory. The techniques such as capture by nets, transportation, freshwater to seawater transfer, and viceversa, grading, sorting, overcrowding, disease treatment etc. results in several types of stress which induce struggling, hypoxia, temperature, and osmotic shock etc. (Mazeaud 1977). Stress can also result as a consequence of other environmental factors such as adverse conditions of temperature, salinity, pH or dissolved oxygen and pollutants (Rosa Flos et al., 1988).

Any form of stress invariably generates stress responses in animals (Mazeaud, 1977). Acute disturbances result in marked physiological stress responses, and these responses have been widely used as indicators of the degree of stress experienced (Barton et al., 1980; Donaldson, 1981; Wedemeyer and McLeay, 1981). A variety of biochemical measurements including blood glucose, and lactate content have been proposed as reliable indices of the relative severity of different stressors in fish and other aquatic invertebrates (Wedemeyer and Yesutake, 1977).

So far the studies related to the stresses are mainly concentrated in the higher groups of animals such as mammals

and fishes. Black et al. (1955) conducted a series of extensive studies on salmonid fishes to understand the pattern of changes in carbohydrate metabolism during severe exercise. Mazeaud et al., (1977) reviewed on primary and secondary effects of stress in fish in general. Later, Rosa Flos et al. (1988), studied the primary and secondary stress responses to grading and hauling in Rainbow trout, Salmo gairdneri.

Stress studies in crustaceans are relatively very few. Most of the literature on stress effects relate to hypoxia or anoxia on the metabolism. (Taylor, 1977; Bridges and Brand, 1979; Burke, 1979). Investigations analysing the metabolic changes during and after severe exercise are still scanty. A few studies analysing the metabolism during and after severe muscular work are concerned with brachyurans (Burke, 1979; McMohan et al., 1979).

In recent years considerable interest has centered around the possible use of the blood sugar level as an indicator of various forms of stress (Huttingh, 1976). It has long been known that different kinds of stress (anoxia, struggling) induces an elevation of blood sugar level in elasmobranchs (Scott, 1921), and teleosts (McCornick and McLeod, 1925). The various procedures involved in

aquaculture practices such as capture and handling almost invariably result in hyperglycemia (Chavin & Yonge, 1970; Wedemeyer, 1972; Miles et al., 1974; Specker and Schreck, 1980). According to Love (1980) the most characteristic general response to stress from whatever source is, a pronounced rise in blood sugar level. Blood glucose concentration have been measured for several cultured cold water fishes particularly salmonids to evaluate the trauma associated with various culture procedures (Donaldson, 1987).

Glycogen serves as the immediate source of energy for intense muscular work in all animals. Meyerhof and Lohman (1928) demonstrated the overall conversion of glycogen to lactic acid in crustacean muscle. It is shown in fishes that the muscle glycogen breaks down during severe exercise (Miller et al., 1959; Black et al., 1965). Nakano and Tomlinson (1967) showed that in Salmo gairdneri, the liver and skeletal muscle glycogen decreased in response to physical disturbances and increased during recovery.

Studies conducted by Prichard & Eddy (1979) on two species of mud shrimp Callianassa californiensis and Upogebia pugettensis exposed to anoxia showed no significant reduction in carbohydrate reserves in muscle and hepatopancreas.

One of the primary chemical mechanism that provides energy for muscular contraction is the anaerobic glycolysis of carbohydrate to lactic acid. Energy demand during the stress of activity often induces anaerobic metabolism causing lactic acidosis. Extensive studies by Black and his colleagues (1955) with several salmonid species have shown that muscle glycogen is rapidly mobilized during exercise and substantial amounts of lactic acid appear immediately in the muscle (Black et al., 1959, 1960, 1961; Parker and Black 1959).

Although, in general a tendency to form lactate is much less in invertebrate tissues compared to vertebrates (Hammen, 1969), there is evidence that crustaceans accumulate lactate under stress conditions. Teal and Carey (1967) showed accumulation of lactate in the marsh crab Uca pugilator under conditions of hypoxia. Studies on lactic acid accumulation during active muscular work were conducted on seven species of crustaceans (Philips et al., 1976). Lactate build up as a result of exercise has been observed in the Australian freshwater yabbie, Cherax destructor (Philips et al., 1977) and in stripped shore crab Pachygraspus crassipes (Dendiger & Schatzlein, 1973). Bridges and Brand (1980) studied the effect of hypoxia and oxygen consumption on blood lactate levels of some marine crustacea. Studies

were carried out on energy metabolism in the tail muscle of shrimp Cragnon cragnon during work and subsequent recovery (Thomas Onnan & Zebe, 1982). Spots & Lutz (1980) established the large and rapid accumulation of lactic acid in the body tissues of two commercially important prawns, Macrobrachium rosenbergii and Penaeus duorarum.

The consequences of stress on lipid metabolism is far from clear. Not many studies are undertaken on this aspect. Recently, more attention has been paid on lipid metabolism in fish which has entailed research in the effect of stress on the free fatty acid (Bilinski, 1974). Mazeaud (1973) showed that stresses which raise the level of blood glucose in Cyprinus carpio, automatically reduce the free fatty acids in the blood.

Till now there are no comprehensive studies on the relative significance of protein metabolism during stress conditions.

There is a global interest in the culture of penaeid prawns to augument production, obviously because of certain special qualities inherent in them. They are euryhaline species, hardy in nature and grows faster to a very large size. They also command a very high price in the international market.

In any large scale culture operation, one of the foremost requirement is the availability of seed as and when required by the farmer. So it is necessary to consider the appropriate infrastructural facilities required for massscale production of prawn seed. Hatcheries play a very important role in the production and steady supply of prawn seeds, thrbughout the season. Whether the seed is produced in the hatchery or collected from the wild they are to be transported and distributed in the farm site. This necessitates transport of seed for shorter or longer durations depending on the distance of the farm site from the seed production or collection centre. Several biological, environmental, and physical factors influence the survival and well-being of the seed. Handling, confinement, oxygen deficiency, high temperature, are some of the very important factors which affect the survival of the seed during transportation.

In many of the commercially important penaeid prawns it is shown that severe physical disturbances during capture and transportation will lead to opaqueness in the abdominal musculature (Rigdon & Baxter, 1979), commonly called as muscle necrosis. When shrimps are exposed to stressful conditions, such as low oxygen, over crowding, or violent movement, the muscles lose their normal transparency and become blotched with whitish areas throughout. Ridgon &

Baxter (1970) studied the histopathological aspects of necrotic muscles in brown shrimp Penaeus aztecus, but could not determine the cause of necrosis. Lekshmi et al., (1977) studied the effect of salinity and temperature changes on spontaneous muscle necrosis in Penaeus aztecus.

A variety of biochemical measurements have been proposed, to evaluate the trauma associated with various culture procedures, such as capture, handling, transportation etc. Several studies have shown that marked species differences exist in the magnitude and duration of biochemical stress responses to various stressors (Wedemeyer, 1976). Thus it is important to examine the stress responses of each species reared in captivity.

The present work was carried out to evaluate the variations in biochemical constituents of haemolymph (glucose, lactic acid, lipid, protein) and muscle (glycogen, lactic acid, lipid and protein) of Indian white prawn Penaeus indicus. Histological observations of the muscle and hepatopancreas of the normal and stressed prawns were also made.

MATERIALS AND METHODS

For the present study, periodic collections were made from farm reared Penaeus indicus, 100-120 mm in size from Matsyafed farm of Kerala State Fisheries Department. Normal healthy animals were selected and transported to the laboratory. On reaching the laboratory the prawns were transferred to large fibre glass tanks of 2 tonne capacity containing filtered and well aerated seawater of 15‰ salinity. The animals were acclimatised to the laboratory conditions for a period of 48 hours before using them for experimental purpose.

Prawns in the intermoult stage were isolated and kept separately for the experiment. In the first experiment strenuous exercise was performed manually on two prawns at a time by continuous chasing and frightening them for a period of 30 and 50 minutes separately. In the second experiment, prawns numbering around 8 to 10 after 30 minutes of strenuous exercise were kept separately for 24 hours in well aerated tanks to recover from the strenuous exertion. Control animals of intermoult stage were also maintained separately without any disturbances and stress. Temperature in the holding tanks during experimental period ranged from 25-27°C, and salinity ranged from 12‰

to 15%. At the end of the stress period, haemolymph samples from all individual prawns were collected. Samples of muscle tissue were also extracted for biochemical analysis.

BIOCHEMICAL ANALYSIS

Biochemical analysis were carried out for quantitative determination of glycogen, lactic acid, lipid and protein in muscle tissue and glucose, lactic acid, lipid and protein content in haemolymph.

HAEMOLYMPH COLLECTION:

Prior to sacrificing the animals haemolymph collections were made from individual prawns through the pericardial cavity using 1 ml. hypodermic glass syringe fitted with number 22 needle. A 5% solution of trisodium citrate was effectively used as an anti-coagulant. The syringe was rinsed with the anticoagulant before extraction of haemolymph. Prior to that the carapace and adjacent areas of individual prawn were dried with absorbant paper to remove the excess moisture adhering to the body. Haemolymph samples were then collected in small glass vials and kept frozen until used.

TISSUE COLLECTION:

After the extraction of haemolymph, the prawns were dissected out immediately, and the fresh tissue such as body muscle and hepatopancreas were excised out, for biochemical and histological work.

Protein content in the muscle and haemolymph was determined by Biuret method (Gornall et al., 1949). Bovine serum albumin (Sigma Chemical Ltd) was used to prepare the standard curve for total protein estimation.

Glycogen content in the muscle tissue was determined by the method of Kemp and Kits (1954). D-Glucose was used for preparing standard curve. The standard curve values were multiplied by 0.927 a conversion factor for glucose to glycogen.

Total lipid content in both muscle and haemolymph were determined by the modified method of 'Sulphophosphovanillin' of Barnes and Blackstock (1973). Extra pure cholesterol was used for the preparation of standard curve.

For the estimation of glucose in haemolymph, the method of Nelson (1944) and Somogyi (1945) was followed. D-glucose was used as a standard for the preparation of standard curve.

Lactic acid content in the muscle and haemolymph was estimated following the method of Barker and Sammerson (1941).

HISTOLOGICAL STUDIES:

Histological studies of muscle tissue and hepatopancreas of normal and stressed prawns were made under the light microscope. Immediately after each experiment the tissues were collected from the animals and then they were fixed in suitable fixatives viz., Bouin's fluid and 10% Neutral buffered formalin.

After 48 hours of fixation, the tissues were washed in running tap water for at least 4 hours and then directly transferred to 70% alcohol for further processing.

Processing of tissues and staining:

For cutting sections of tissues in paraffin, the dehydration and clearing of the tissues were carried out at room temperature (28°C). The tissues were dehydrated through ascending series of alcohol grades starting from 70% upto 100%. The tissues were kept in each grade for at least one hour before passing to the other grade. After dehydration is completed the tissues are cleared through 3 changes of chloroform. Before embedding the tissues in

paraffin wax, one hour impregnation in molten wax (56-58°C M.P.) was given twice.

The .. sections were cut at 4 to 6 μ thickness using a manual rotary microtome. After deparaffining in xylene the sections were hydrated through descending grades of alcohol, washed finally in distilled water and then stained with Harris alum Haematoxylin, and counterstained with 1% alcoholic eosin. The stained sections were then dehydrated and mounted in DPX through xylene. Photomicrographs of the histological preparations were taken using American optical Research Microscope.

Statistical analysis was done to find out any significant differences in the experimental results. All the statistical analysis were carried out according to method of Snedecor and Cochran (1967).

PLATE I

Plate 1. Normal (A) and stressed (B) *Penaeus indicus*. White foci (C) on first four abdominal segments show different stages of necrosis development.

R E S U L T S

The results of the estimation of biochemical constituents of haemolymph and muscle tissue of the prawn, *Penaeus indicus* after 30 and 50 minutes strenuous exercise and for the recovery period are presented in table 1 and table 2.

BIOCHEMICAL CONSTITUENTS

1. HAEMOLYMPH

Glucose:

Glucose content in haemolymph showed a rapid increase after 30 and 50 minutes strenuous exercise (46.64 ± 0.2 mg% and 48.2 ± 4.5 mg% respectively). In normal healthy animals the glucose content was found to be in the range 11-26 mg%. During the recovery period of 24 hours after subjecting the prawns for strenuous exercise, the blood glucose content almost regained its normal value (18 mg%) (Fig.1).

Statistical analysis was carried out using Analysis of variance (ANOVA) and the differences in the glucose content of haemolymph during different stages of stress conditions are found to be statistically significant, at 5% level. (Table 3 a).

Lactic acid:

Strenuous exercise resulted in a large and rapid rise in the concentration of lactic acid in haemolymph (Fig. 2). The average blood lactate value in unexercised normal prawns was 30.89 ± 9.26 mg%. After 30 minutes strenuous activity the blood lactate level rose to 56.04 ± 9.45 mg %. In the stressed and exhausted prawns (50 minutes strenuous exercise) the value was 77.32 ± 13.2 mg%. After 24 hours of recovery the lactate level almost reached the normal level (47.64 ± 9.7 mg%).

ANOVA showed that the difference in lactic acid content of haemolymph between the normal, stressed, and stress recovered prawns are statistically significant (Table 3b).

Lipid:

Lipid level in haemolymph showed a slight decrease during stress conditions (Fig. 3). After 30 minutes of continuous physical exercise the lipid level showed a marked decrease from normal value and the values observed were 253.72 ± 30.97 mg% and 163.8 ± 21.06 mg % respectively. It showed a further decrease in 50 minutes stressed prawns (138.67 ± 16.99 mg%). The stressed (30 minutes) and recovered animal showed an almost normal level with an average of 219.8 ± 26.77 mg%.

ANOVA indicated that the difference in the lipid content of haemolymph between normal, stressed and stress recovered prawns are statistically significant (Table 3c).

Protein:

Protein content in haemolymph did not show any significant change between normal and stressed prawns (Fig. 4). The average haemolymph protein content in control animal was found to be 5.6 ± 0.9309g%. There was no significant change in haemolymph protein content after 30 and 50 minutes strenuous exercise 4.94 ± 0.684 mg% and 4.76 ± 0.6066g% respectively. During the recovery phase the protein content was found to be 4.83 ± 0.53g%.

Results of the ANOVA revealed that differences in the protein content of haemolymph between the normal, stressed and stress recovered prawns were not significant statistically. Table (3d).

2. MUSCLE TISSUE

Glycogen:

Muscle glycogen showed a significant reduction after the application of strenuous physical exercise (Table 2; Fig. 5). After 30 minutes of strenuous exercise, the muscle glycogen value was found to be 0.126 ± 0.35 mg%,

more than 50% reduction from the normal value (0.32 ± 0.13 mg%). After 50 minutes of strenuous exercise, the level further fell down to 0.073 ± 0.03 mg%. In the recovery phase however the glycogen level was not found to be restored and the average value observed was 0.096 ± 0.038 mg%.

ANOVA showed that the differences in glycogen content of muscle between normal, stressed and stress recovered prawns are statistically significant (Table 4a).

Lactic acid:

As in the case of haemolymph lactate, muscle lactate also showed a rapid increase during strenuous exercise (Fig. 6). After 30 minutes of strenuous exercise, muscle lactate showed a steep rise from a control value of 0.093 ± 0.025 mg% to 0.262 ± 0.047 mg%. The lactate level further increased upto 0.402 ± 0.059 mg%, after 50 minutes of strenuous exercise. During the recovery phase, the muscle lactate level came down and was seen slightly higher than the normal value (0.168 ± 0.23 mg%).

Results of ANOVA revealed that these differences in muscle lactate content between normal, stressed, and stress recovered animals are statistically significant (Table 4b).

Lipid:

Muscle lipid level showed a slight decrease during the stress period (Fig. 7). After 30 minutes of strenuous exercise, the lipid value came down from a normal level of 2.95 ± 0.32 mg/100 mg to 2.04 ± 0.646 mg%. Lipid level showed a further decrease after 50 minutes of strenuous exercise (1.704 ± 0.857 mg%). Stress recovered prawns showed an average lipid content of 2.477 ± 0.382 mg%.

Anova showed that the differences in lipid content of muscle between normal, stressed and stress recovered prawns are statistically significant (Table 4c).

Protein:

Protein content in the muscle exhibits no significant variation between normal and strenuously exercised prawns. (Fig. 8). The average muscle protein content in normal animals was 14.75 ± 1.03 mg%. After 30 and 50 minutes exercises the protein content showed no significant variation, the values being 14.2 ± 0.55 mg%, and 15.2 ± 0.885 mg% respectively. During the recovery period, the protein content was found to be 15.1 ± 0.877 mg%.

ANOVA showed no significant variation in muscle protein content in normal, stressed, and stress recovered prawns (Table 4d).

Table 1: Variations in biochemical constituents in haemolymph of *P. indicus*, after 30 and 50 minutes of strenuous exercise and for the recovery period of 24 hours.

Haemolymph (mg/100 ml)		Normal unexercised	Strenuous stress 30 minutes	Strenuous stress 50 minutes	24 hrs. recovery after 30 minutes severe stress
Glucose	Mean	13.75^{a}	46.64^{b}	48.2^{b}	18.201^{a}
	SD	± 2.12	± 7.23	± 4.50	± 4.67
	Range	11 - 18	37 - 59	44 - 56	13 - 26
Lactic acid	Mean	30.89^{c}	56.04^{b}	77.316^{d}	47.648^{c}
	SD	± 9.26	± 9.415	± 13.2	± 9.722
	Range	15 - 40	59 - 80	55 - 88	33 - 55
Lipid	Mean	253.72^{a}	161.88^{b}	138.66^{c}	219.8^{a}
	SD	± 30.97	± 21.06	± 16.99	± 26.76
	Range	213 - 280	133 - 193	120 - 160	200 - 266
Protein	Mean	5.6^{b}	4.94^{b}	4.76^{b}	4.83^{b}
	SD	± 0.93	± 0.686	± 0.606	± 0.521
	Range	4.6 - 7.0	4 - 6	4 - 5.6	4 - 5.6

All values are mean of 8 determinations $\bar{X} \pm$ SD.

"Mean values with the same superscription do not differ significantly".

Table 2: Variations in biochemical constituents in muscle tissue of *P. indicus*, after 30 and 50 minutes of strenuous exercise and for the recovery period of 24 hours.

Muscle tissue (mg/100 mg)		Normal unexercised	Strenuous stress 30 minutes	Strenuous stress 50 minutes	24 hrs. recovery after 3[illegible] minutes severe stress
Glycogen	Mean	0.321^{a}	0.126^{b}	0.0732^{c}	0.0962^{c}
	SD	± 0.1289	± 0.035	± 0.030	± 0.0375
	Range	0.201 - 0.486	0.088 - 0.180	0.034 - 0.134	0.061 - 0.160
Lactic acid	Mean	0.0933^{m}	0.2619^{n}	0.402^{o}	0.168
	SD	± 0.0248	± 0.0975	± 0.0599	$\pm$ 0.0446
	Range	0.066 - 0.144	0.210 - 0.324	0.322 - 0.445	0.097 - 0.225
Lipid	Mean	2.954^{a}	2.04^{b}	1.7039^{c}	2.9766^{a}
	SD	± 0.320	± 0.646	± 0.8569	± 0.3817
	Range	2.37 - 3.37	1.068 - 2.90	0.098 - 3.180	2.136 - 2.88
Protein	Mean	14.75^{b}	14.2	15.2^{b}	15.1^{b}
	SD	± 1.030	± 0.555	± 0.855	± 0.872
	Range	14 - 16.4	13.6 - 15	14 - 16.4	14 - 16.8

All values are mean of 8 determination $\bar{X}$ & SD.

"Mean values with the same superscription do not differ significantly.

Fig. 1. Changes in glucose content of haemolymph after 30 and 50 minutes of strenuous exercise and a recovery period of 24 hours.

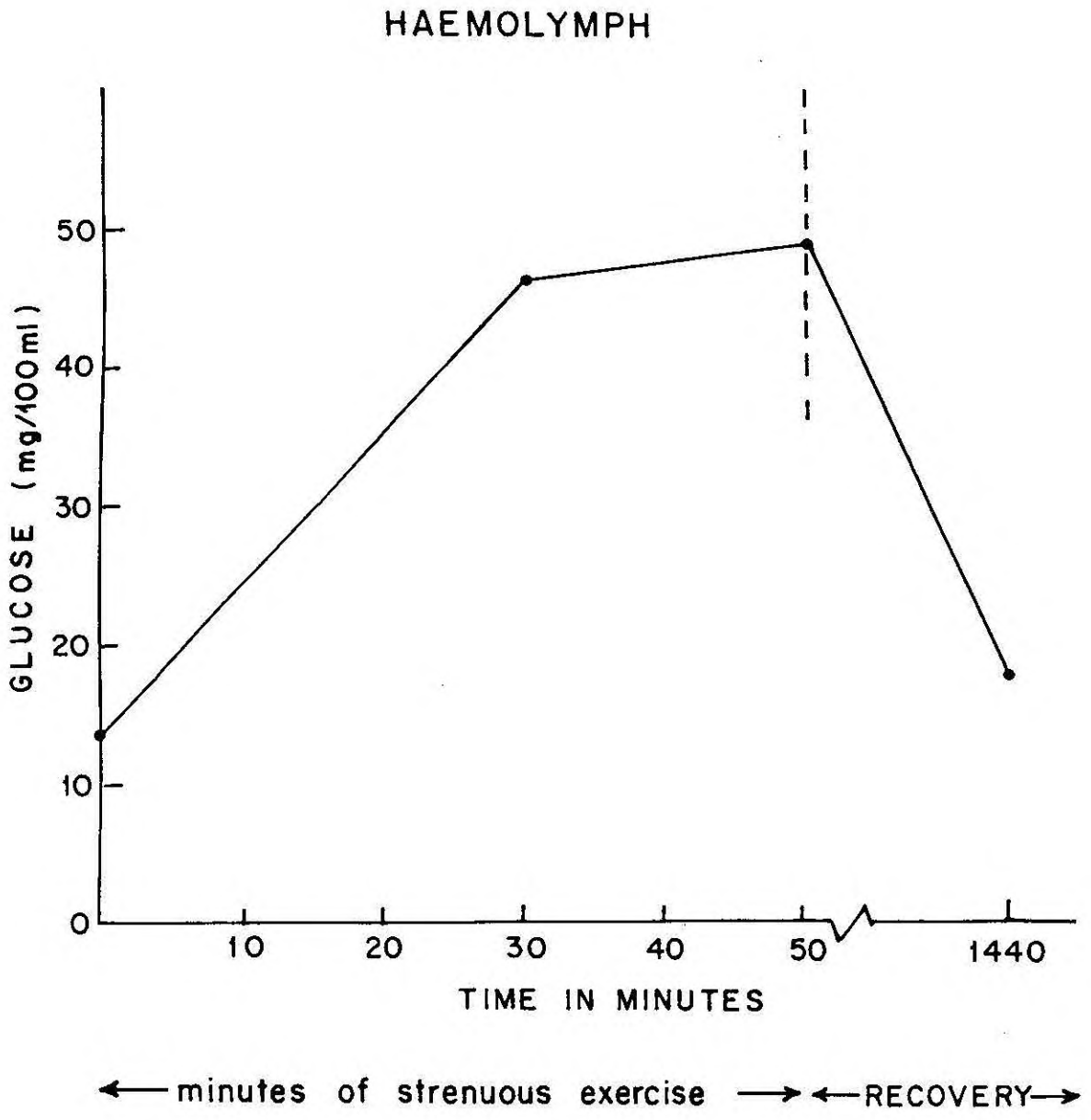

HAEMOLYMPH
GLUCOSE (mg/100ml)
50
40
30
20
10
0
10
20
30
40
50
1440
TIME IN MINUTES
← minutes of strenuous exercise →
←RECOVERY→

Fig. 2. Changes in lactic acid content of haemolymph after 30 and 50 minutes of strenuous exercise and a recovery period of 24 hours.

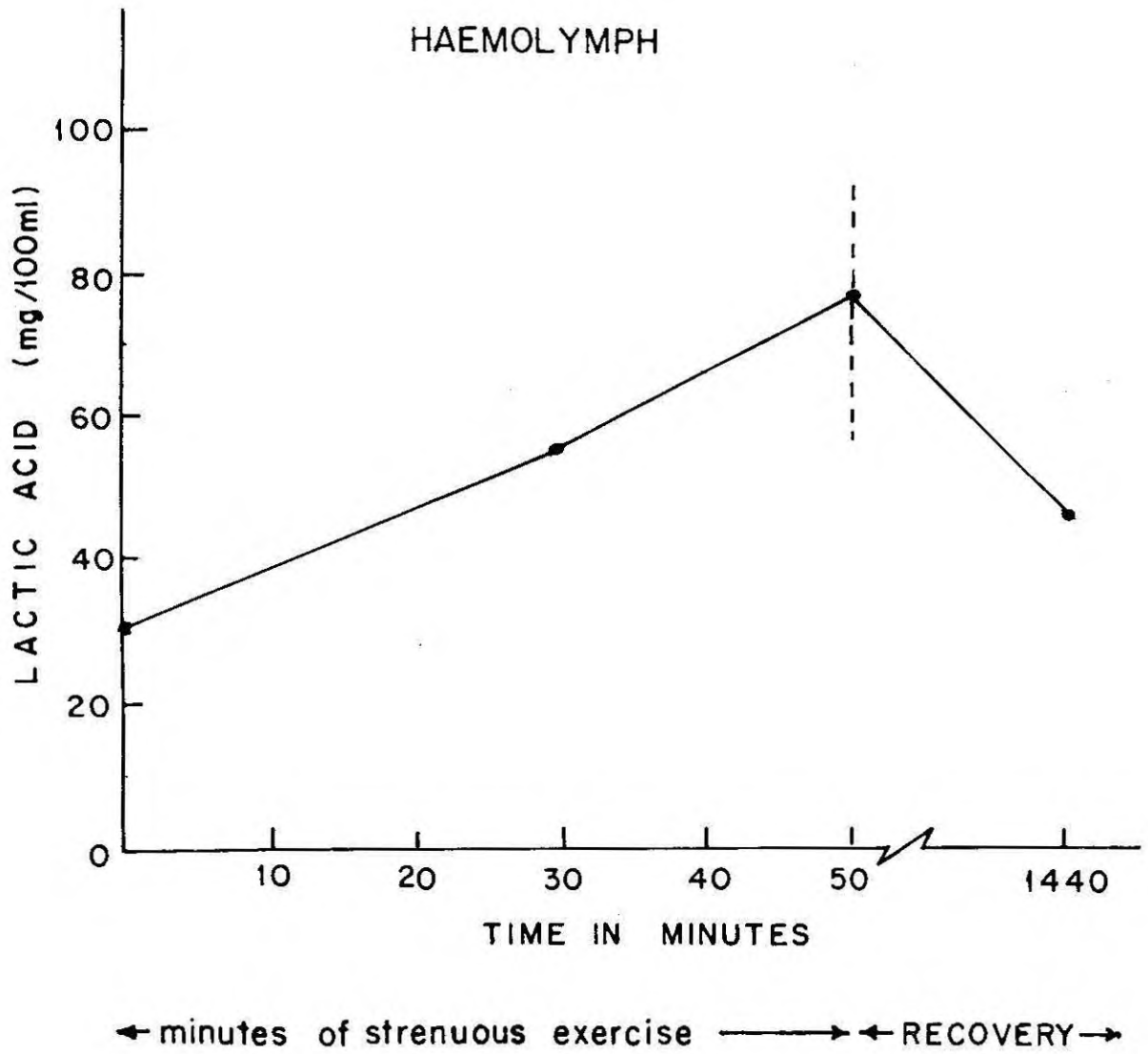

HAEMOLYMPH
LACTIC ACID (mg/100ml)
100
80
60
40
20
0
10
20
30
40
50
1440
TIME IN MINUTES
minutes of strenuous exercise
RECOVERY

Fig. 3. Changes in lipid content of haemolymph after 30 and 50 minutes of strenuous exercise and a recovery period of 24 hours.

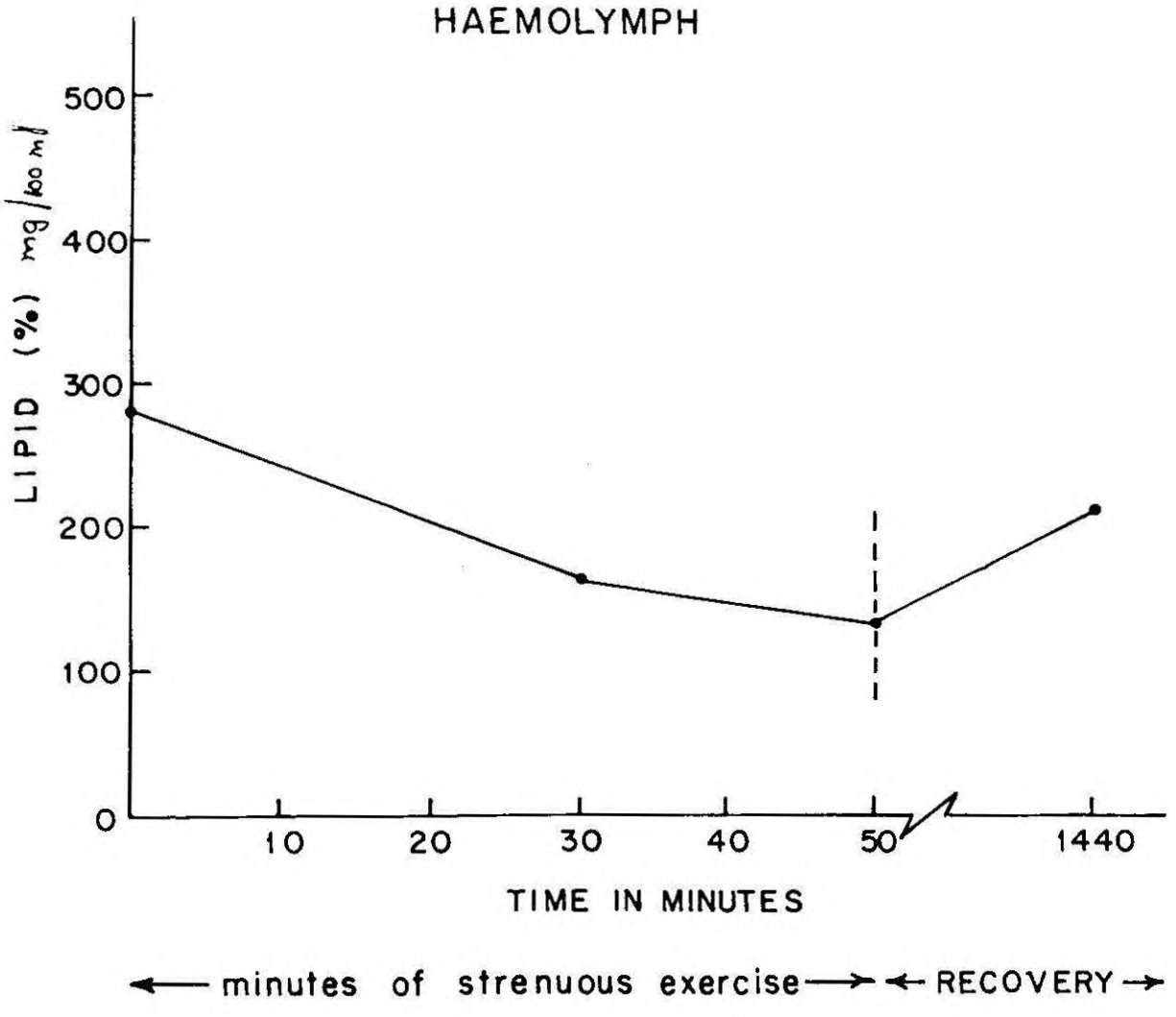

HAEMOLYMPH
LIPID (%) mg/100 ml
500
400
300
200
100
0
10
20
30
40
50
1440
TIME IN MINUTES
minutes of strenuous exercise
RECOVERY

Fig. 4. Changes in protein content of haemolymph after 30 and 50 minutes of strenuous exercise and a recovery period of 24 hours.

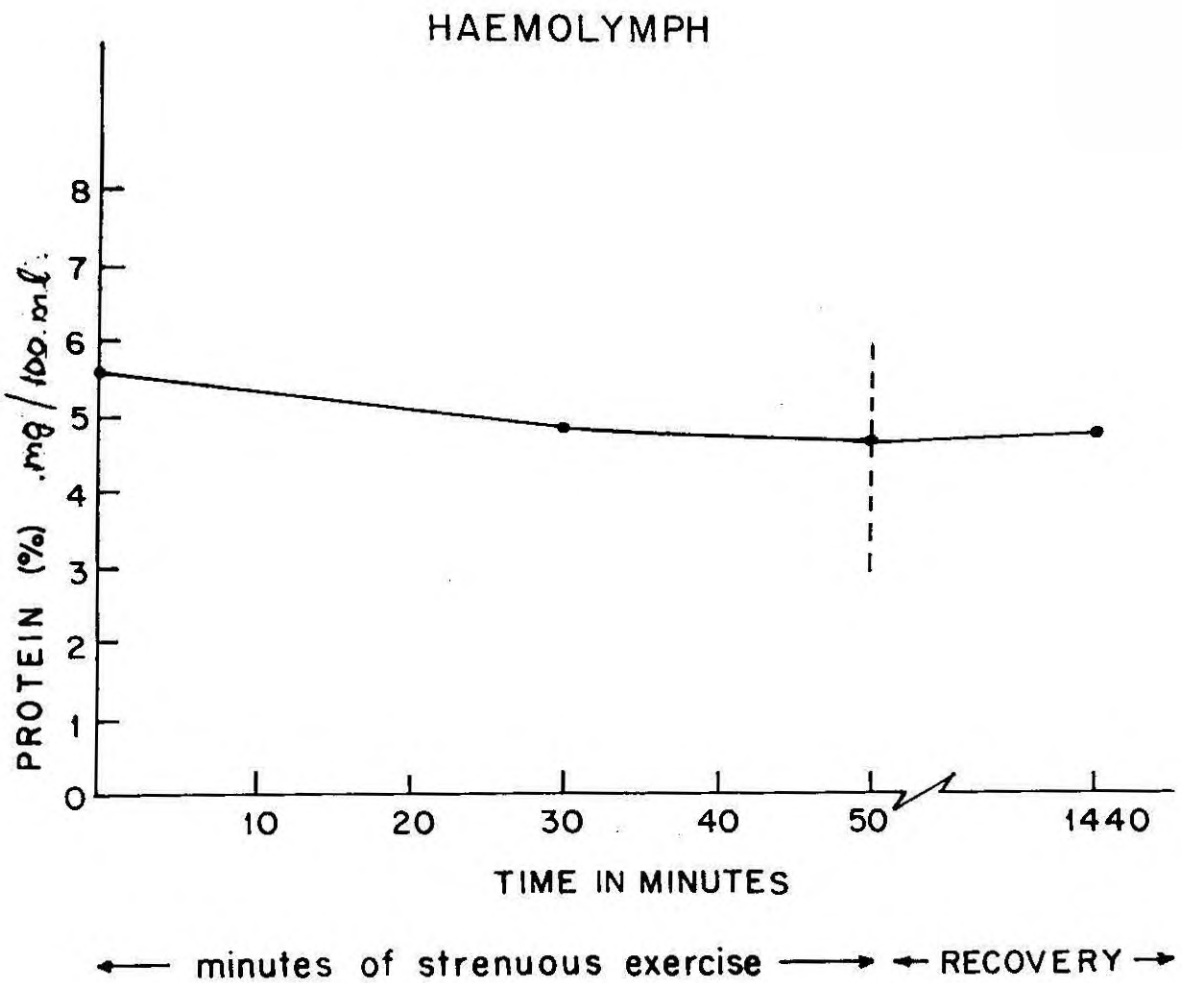

HAEMOLYMPH
PROTEIN (%) mg/100 ml
0
1
2
3
4
5
6
7
8
10
20
30
40
50
1440
TIME IN MINUTES
minutes of strenuous exercise
RECOVERY

Fig. 5. Changes in glycogen content of muscle after 30 and 50 minutes of strenuous exercise and a recovery period of 24 hours.

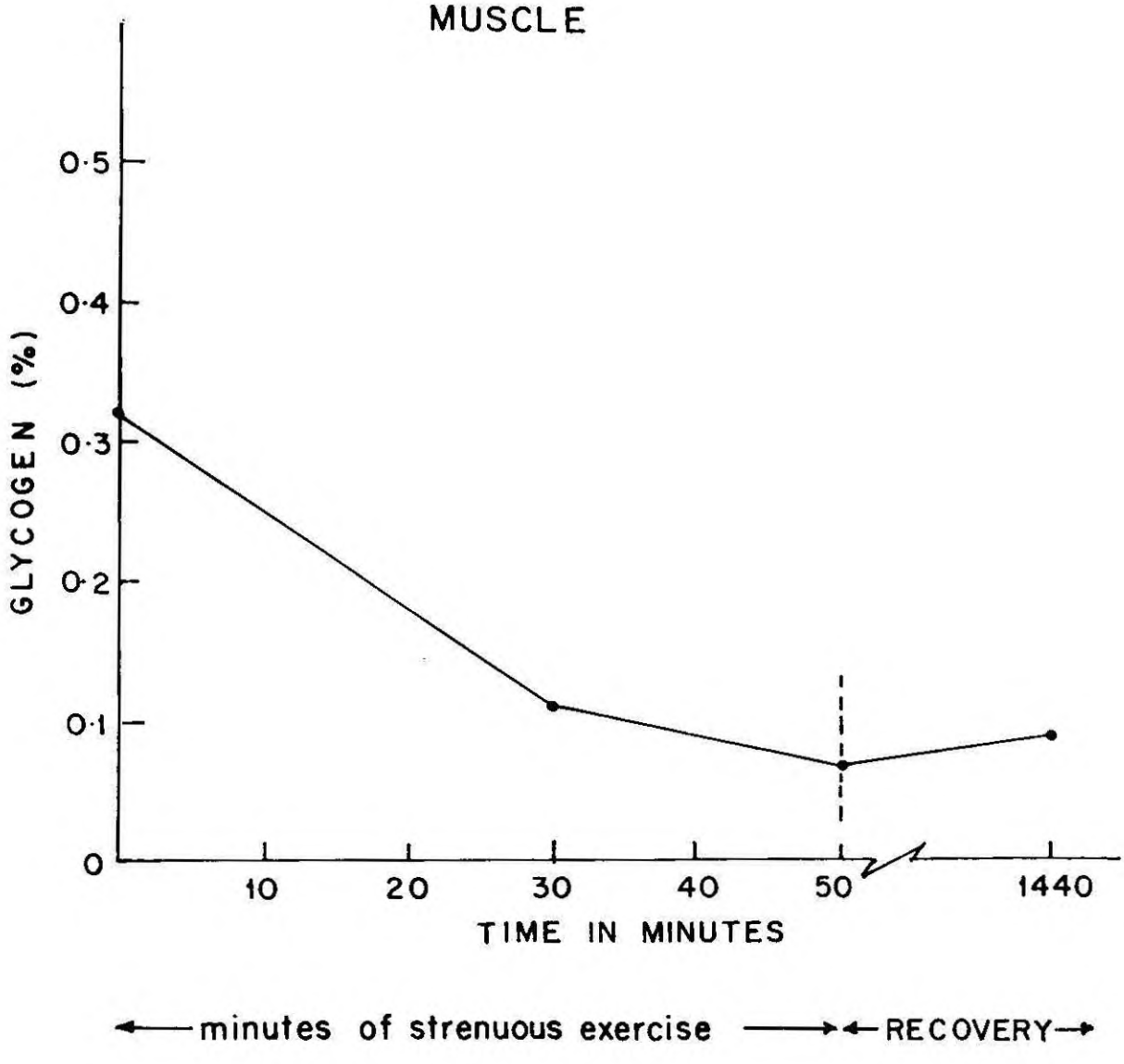

MUSCLE
GLYCOGEN (%)
0·5
0·4
0·3
0·2
0·1
0
10
20
30
40
50
1440
TIME IN MINUTES
minutes of strenuous exercise
RECOVERY

Fig. 6. Changes in lactic acid of muscle after 30 and 50 minutes of strenuous exercise and a recovery period of 24 hours.

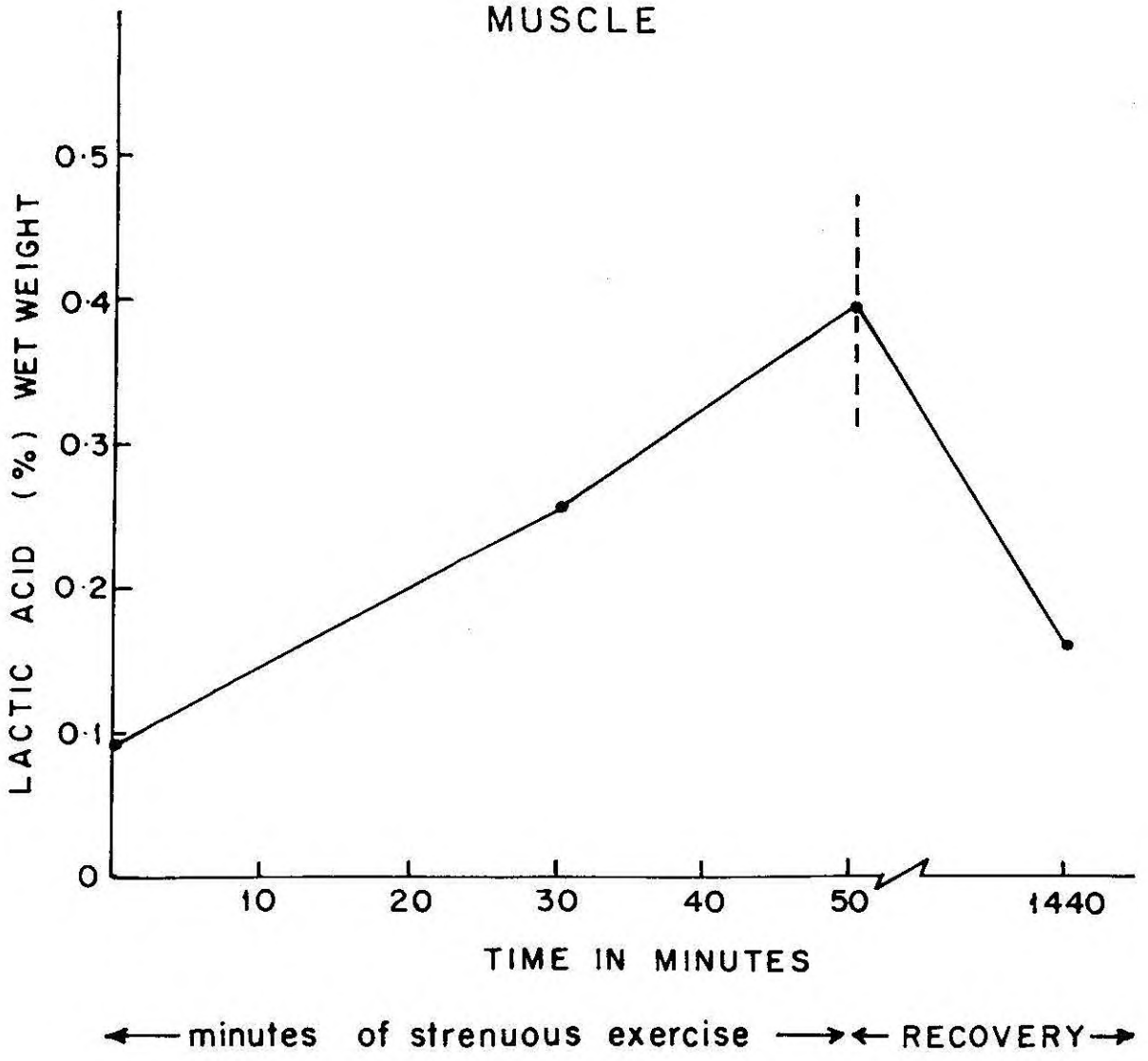

MUSCLE
LACTIC ACID (%) WET WEIGHT
0·5
0·4
0·3
0·2
0·1
0
10
20
30
40
50
1440
TIME IN MINUTES
minutes of strenuous exercise
RECOVERY

Fig. 7. Changes in lipid content of muscle after 30 and 50 minutes of strenuous exercise and a recovery period of 24 hours.

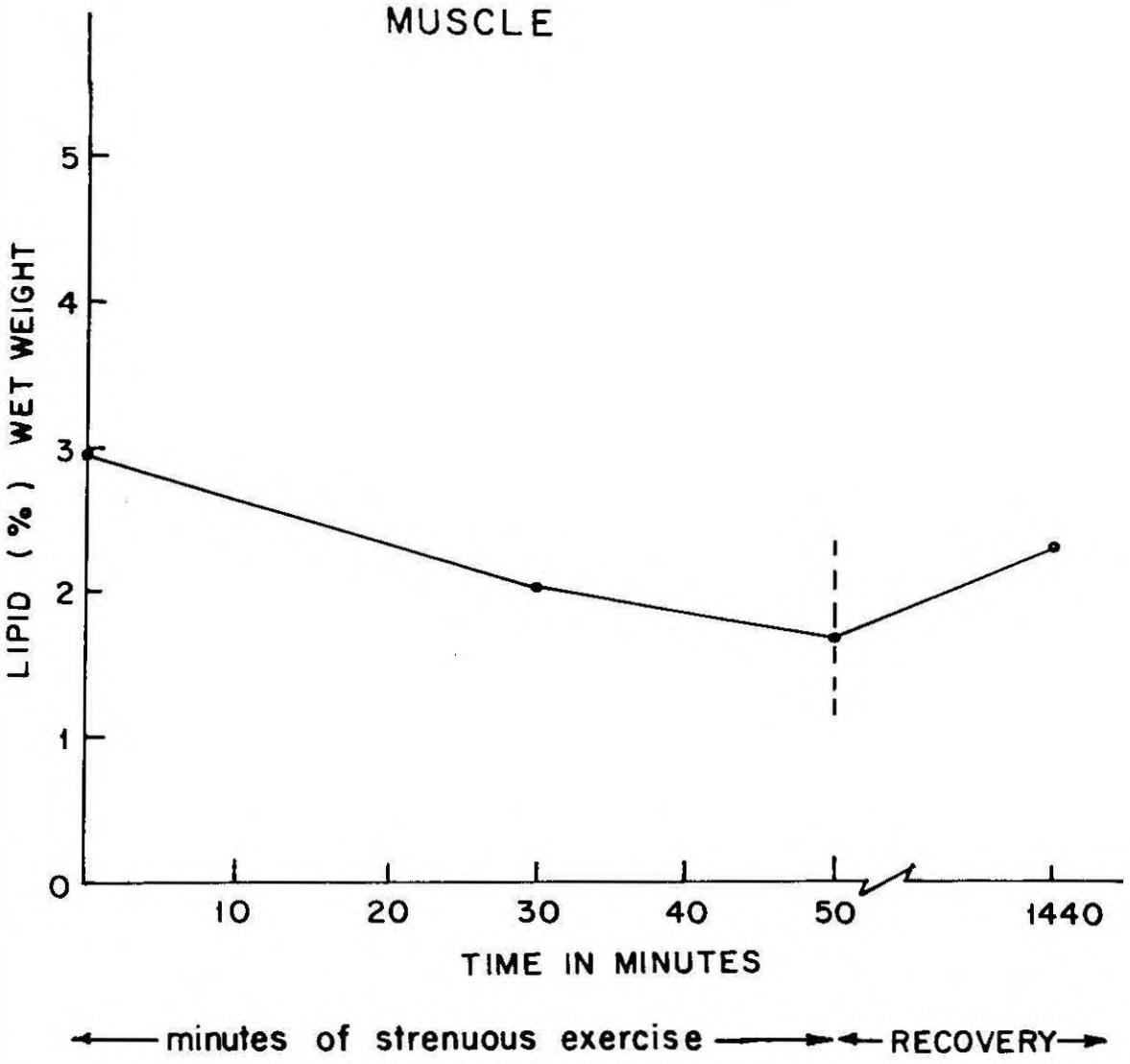
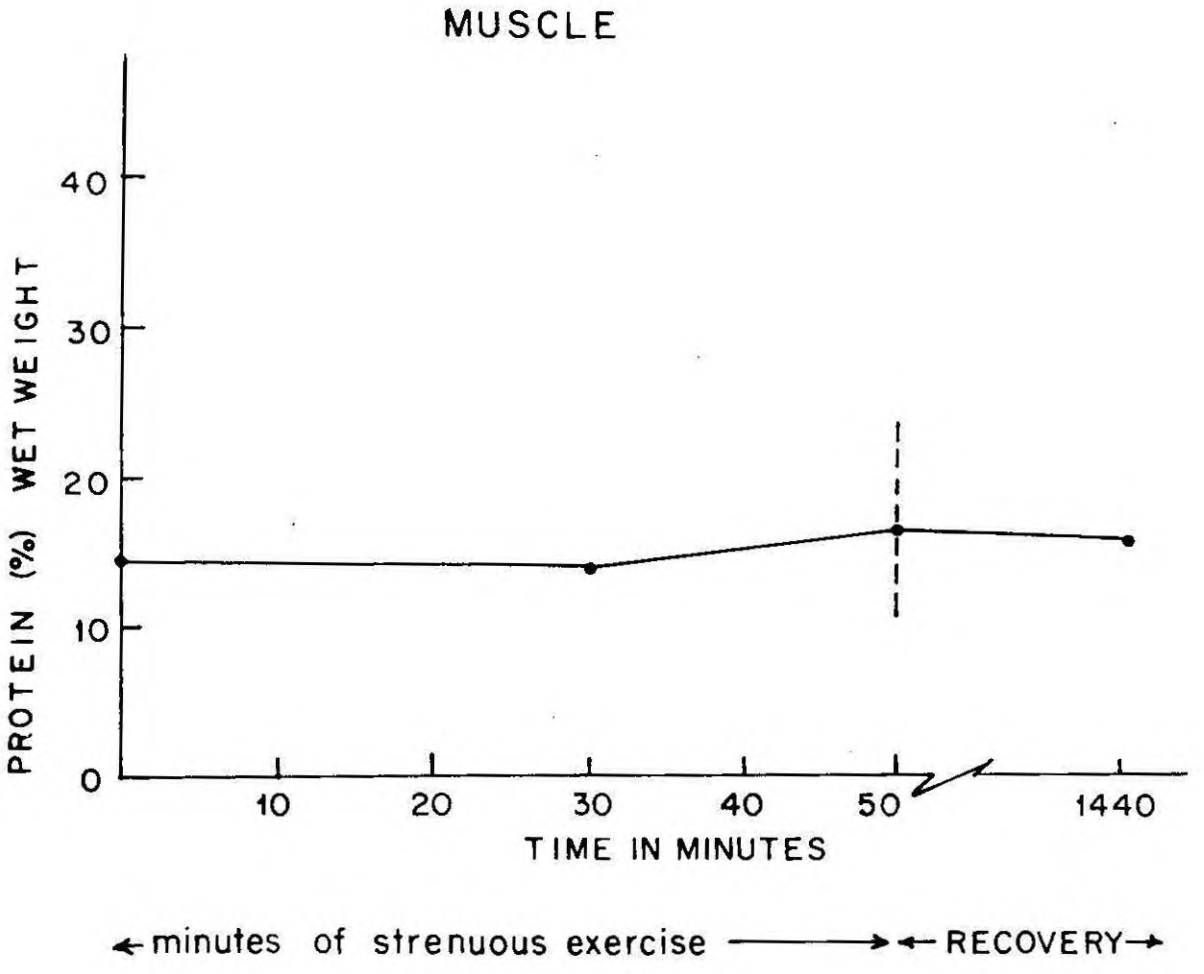

MUSCLE
LIPID (%) WET WEIGHT
0
1
2
3
4
5
10
20
30
40
50
1440
TIME IN MINUTES
minutes of strenuous exercise
RECOVERY

Fig. 8. Changes in Protein content of muscle after 30 and 50 minutes of strenuous exercise and a recovery period of 24 hours.


MUSCLE
PROTEIN (%) WET WEIGHT
0
10
20
30
40
10
20
30
40
50
1440
TIME IN MINUTES
← minutes of strenuous exercise →
← RECOVERY →

STATISTICAL ANALYSIS

ANOVA TABLES

Table - 3a. Haemolymph glucose

Source	D.F.	Sum Sqr	Mean Sum Sqr	F.Value	Remarks
Treat	3	7436.9	2478.97	100.11	HIG.SIG (1%)
Error	26	643.8	24.76		

Table - 3b. Haemolymph lactate

Source	D.F.	Sum Sqr	Mean Sum Sqr	F.Value	Remarks
Treat	3	7668.06	2556.02	18.26	HIG.SIG (1%)
Error	19	19.0	2659.6		

STATISTICAL ANALYSIS

ANOVA TABLES

Table 3c. Haemolymph lipid

Source	D.F.	Sum.Sqr	Mean sum Sqr	F. Value	Remarks
Treat	3	49059.5	16353.19	27.48	HIG.SIG (1%)
Error	19	11306.19	595.00		

Table 3d. Haemolymph protein

Source	D.F.	Sum.Sqr	Mean sum Sqr	F. Value	Remarks
Treat	3	4.9409	1.6469	2.97	N.S.
Error	24	13.32	0.555		

STATISTICAL ANALYSIS

ANOVA TABLES

Table 4a. Muscle glycogen

Source	D.F.	Sum Sqr	Mean Sum Sqr	F. Value	Remarks
Treat	3	0.29119	0.0970	6.18	HIG.SIG (1%)
Error	23	7.628	0.33169		

Table 4b. Muscle lactate

Source	D.F.	Sum Sqr	Mean Sum Sqr.	F.Value	Remarks
Treat	3	0.43425	0.14415	62.10	HIG.SIG (1%)
Error	26	0.06036	0.00232		

STATISTICAL ANALYSIS

ANOVA TABLES

Table 4c. Muscle lipid

Source	D.F.	Sum Sqr	Mean Sum Sqr	F. Value	Remarks
Treat	3	6.1517	2.0505	6.18	HIG.SIG (1%)
Error	23	7.628	0.33169		

Table 4d. Muscle protein

Source	D.F.	Sum Sqr	Mean Sum Sqr	F. Value	Remarks
Treat	3	4.5517	1.51725	2.19	N.S.
Error	29	16.6039			

Histological Studies:

Histological observations were made on muscle and hepatopancreas of both normal and stressed prawns.

The normal muscle cell or muscle fibre is surrounded by a plasmamembrane, the sarcolemma, and within it the elliptical nuclei with their long axis parallel to the length of the fibre. The muscle cell consists of thousands of myofibrils. Thus muscle cell is an elongated, multinucleated unit. In the present study prawns are found to develop opaque white patches in the abdominal segments after 30 and 50 minutes of strenuous physical exercise.

Histological observations of the abdominal muscle of stressed prawns revealed that the white opaque areas were necrotic. Areas of necrosis were usually surrounded by normal muscle tissue. Opaque muscle fibres displayed a variety of morphological changes characteristic of progressive segmental myofibre degeneration. The most characteristic change observed in the muscle after 30 minutes of strenuous exercise were a swelling of muscle cell followed by a loss of usual cross striations (Plate II, Fig. 2). Some of the muscle cells showed the presence of pyknotic nuclei.

50 minutes stressed prawns showed a more wide distribution of opaque white patches in the abdominal segments.

Histological observations of these areas showed severe myofibre degeneration. Fusion and cross-splitting of myofibrils were also observed (Plate II: Fig.3). Haemocytic infiltration was observed around the necrotic foci (Plate II: Fig. 2). A prominent shrinkage of the myofibres were also observed at the necrotic foci caused by progressive loss of myofibre parenchyma (myofibrils and sarcoplasm). Areas of myofibre disorganisation were also characterized by numerous single rows, and aggregations of hyperchromatic myonuclei, and pyknotic nuclei. The area of necrosis were distributed randomly throughout the striated musculature of the body.

The bulk of hepatopancreas is composed of a large number of blind-ended finger like tubules, the walls of which were composed of a simple epithelium which contained 4 types of epithelial cells, corresponding to the classification of E-, R-, B-, F-, used by Hirsch & Jacobs (1930). Only two types of epithelial cells were clearly observed in the present study, the secretory cells (F-, B- cells), which are very large and highly basophilic and the absorptive cells (R-cell). Secretory cells are very prominent and were seen to contain one or two large vacuoles. Absorptive cells were long and narrow in shape and appeared to be 'Squeezed' between other cell type, but extended throughout the thickness of the epithelium.

No significant histological changes were observed in the hepatopancreas of prawns subjected to strenuous physical exercise,except an extensive vacuolation in the epithelial cells of the tubule. Frozen sections of hepatopancreas of normal and stressed prawns, stained with oil red showed almost similar pattern in the distribution of lipid in the hepatopancreas.

PLATE II

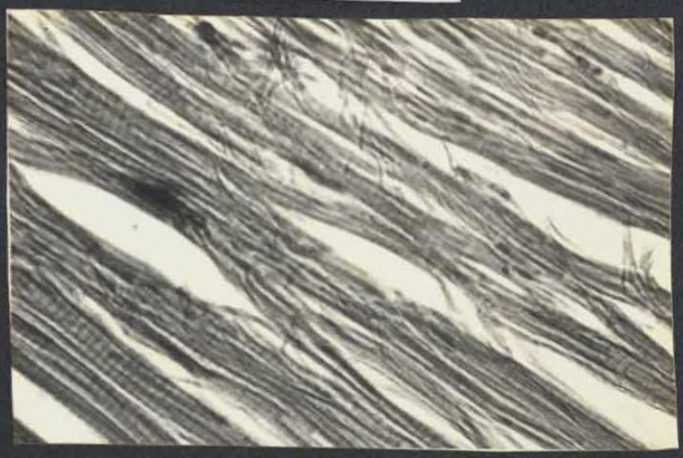


Fig. 1 . Longitudinal section of abdominal muscle of normal prawn. H&E X400

Fig. 2 . L. S. of abdominal muscle of 30 minute stressed prawn showing swollen nature of muscle fibres. H&E X400

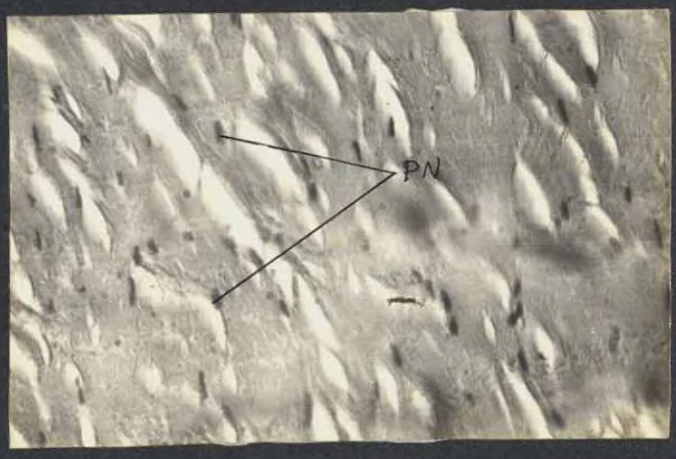


Fig. 3 . Higher magnification of figure 2. Note the loss of cross-striations. H&E X1000.

PLATE III

Fig. 1 . Photomicrograph showing myofibrillar disorganisation. H&E X400

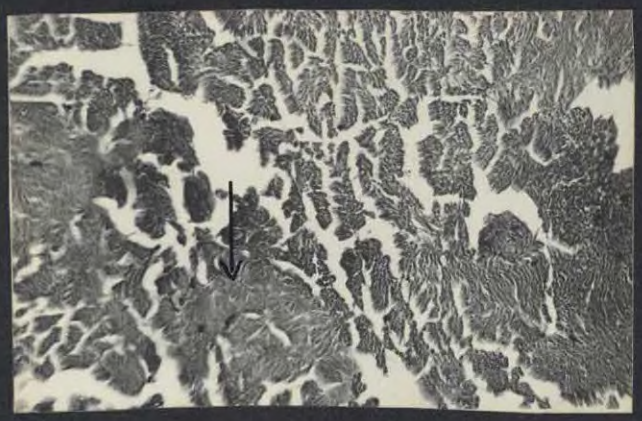


Fig. 2 . T.S. of opaque abdominal muscle showing oedematous condition of muscle tissue. H&E X400.

Fig. 3 . Cross section of the opaque area of abdominal muscle showing degenerating muscle tissue surrounded by normal muscle H&E X400.

PLATE IV

Fig. 1 . T.S. of abdominal muscle of 50 minutes stressed prawn. Note the ground glass appearance of muscle tissue. H&E X400.

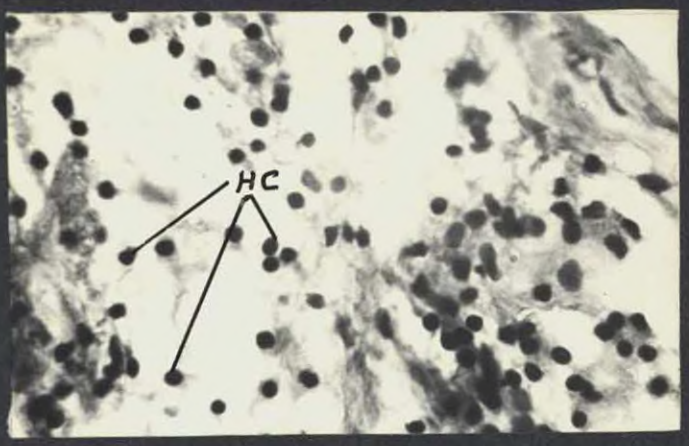


Fig. 2 . Photomicrograph showing intense infiltration of haemocytes (HC) at the necrotic foci. H&E X1000.

Fig. 3 . Note the focal aggregation of haemocytes in necrotic myofibres. H&E X400.

PLATE V

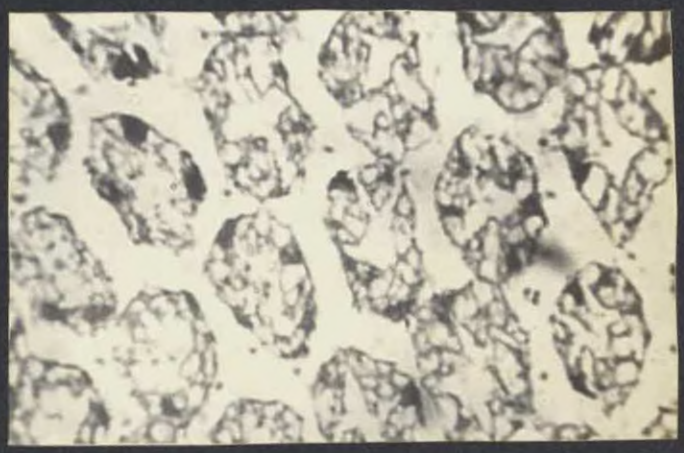


Fig. 1 . Cross section through hepatopancreatic tubule of stressed prawn showing extensive vacuolation in the tubule. H&E X400.

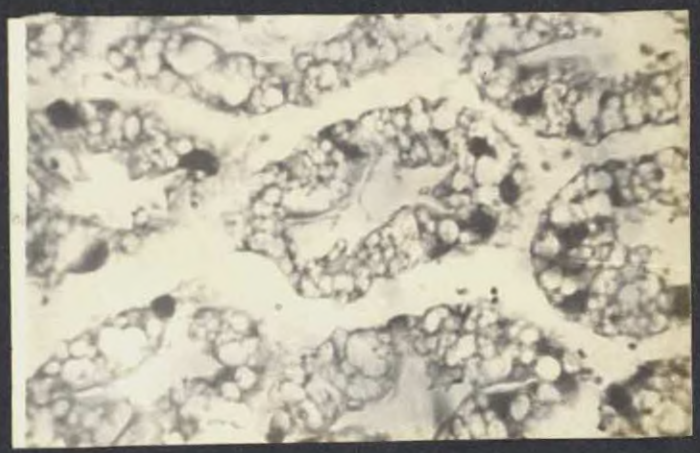


Fig. 2 . Higher magnification of Fig. 1 . H&E X1000.

D I S C U S S I O N

It is a well known fact that handling and other stress causing procedures involved in aquaculture alter the basal metabolite level of animals (Barton et al., 1980). The trauma associated with aquaculture techniques and the adverse conditions of intensive culture system have been evaluated by the measurement of various biochemical stress responses (Wedemeyer 1982; Schreck 1981). The biochemical variables measured include circulating levels of cortico-steroid hormones, glucose, lactic acid, lipids, proteins, electrolyte and liver glycogen. Changes in these variables serve as indicators of the adverse consequence of stressors. (Wedemeyer and Yasutake 1977; Wedemeyer & McLeay 1981). Monitoring of biochemical stress indices in cultured fish and shellfish can be useful for evaluating the trauma associated with different culture procedures and system.

Carbohydrates present in the blood and muscle serve as the immediate source of energy for muscular work. Meyerhof and Lohrman (1928) demonstrated the overall conversion of glycogen to lactic acid in crustacean muscle. Later work proved that glycolysis is the main route of glucose degradation in Crustacea (Hu, 1958; Hochachka et al., 1962; McWhinne et al., 1962; Huggins, 1966). The influence

of stressors on carbohydrate metabolism in fish has received considerable attention (Wedemeyer & McLeay, 1981).

In recent years a great deal of interest has been centered around the possible use of blood sugar level as an indicator of various forms of stress. According to Love (1980), the most characteristic general response to stress from whatever source, is a pronounced rise in blood sugar level, which seems to occur whenever the physical activity exceeds what is normal for the animal. The rise in blood sugar level resulting from stress has now been thoroughly documented. Evidently, an increase in blood glucose level serves as a sensitive indicator of many types of unfavourable environmental conditions, such as capture (Wardle, 1972), handling (Pickering et al. 1982), transportation (Huttingh 1976). Tandon and Joshi (1973) showed that the blood glucose level in Heteroneustes fossilis increased rapidly for the first two hours after stress and rose to 190% of pre-stress value, and further they found that this hyperglycemia returned to normal after 24 hours of recovery. Investigations have been made to show that exercise unconnected with asphyxial condition can also cause a rise in blood sugar (Keirmeir, 1939; Dean, 1962). When exercise is too strenuous or lasts too long the blood sugar can rise to high values. The work of Sibergeld

(1974) suggests that, though the blood sugar level is a sensitive indicator of environmental and other stress, other factors are also involved in a rise of blood sugar and therefore the findings should be used and interpreted with care.

A rise in haemolymph glucose observed in the present study in P. indicus immediately after strenuous physical exercise and decline to normal values after 24 hours of recovery corroborates well with the findings of earlier workers in both fishes and crustaceans (Keirmeir, 1939; Dean 1962).

The elevated levels of blood glucose may be due to increased basal metabolic rate during stress conditions and, further degradation of hepatopancreas or muscle glycogen. Umminger and Gist (1971) showed that stress due to injection in Carassius auratus resulted in an increased serum glucose and a decline in the liver and muscle glycogen. Hayashi and Goshiro (1975) showed that the emergency hormone adrenaline is closely concerned with the stress effects in blood glucose and its injection into Anguilla japonica stimulates glycogenolysis in the liver. They pointed out that this response would provide for the increased glucose supplies needed during the increased muscular activity.

The results of the present study indicated that the metabolism of muscle glycogen in P. indicus is extremely rapid. A more than 50% reduction in muscle glycogen can be seen after 30 minutes of strenuous physical exercise. Probably this metabolism provides most, if not all, of the energy requirement for severe muscular activity. Many of the earlier studies on fishes and crustacean showed similar results (Black et al., 1959; Prichard & Eddy 1979; Onnen & Zebe 1983). Studies on the phosphorylases of fish muscle by Cordier & Cordier (1957) and Ono and Nagayama (1957) showed that all the enzymes necessary to convert glycogen rapidly to lactic acid are present in the skeletal muscle and other tissues. Drummond & Black (1960) showed that though fishes depend on other energy sources such as protein and lipid at times like, during spawning migration, the immediate source of fuel at the cellular level, particularly for burst of strenuous activity may still be the muscle glycogen. The metabolism of lipid and protein is essentially a slow process, hardly rapid enough for sudden spurts of muscle contraction.

Studies conducted by Black (1959) on rainbow trout Salmo gairdneri, showed a rapid depletion of muscle glycogen immediately after exercise. The level became one half the resting level after 2 minutes of strenuous activity.

Restoration of glycogen after 24 hours of recovery was slow and incomplete. A similar situation has been documented in the Plaice (Wardle, 1967).

Teal and Carey (1967) showed accumulation of lactate and concurrent utilization of glycogen in the marsh crab Uca pugilator under conditions of hypoxia. But Prichard & Eddy (1979) could not find any significant depletion in muscle glycogen in their studies on two species of mud dwelling shrimp, Callianassa californiensis and Upogebia pugettensis exposed to anoxia.

The results of the present study have shown that P. indicus accumulates lactate as a result of continuous physical exercise, and indicate that anaerobic metabolism suppliments or replaces aerobic energy production under these conditions. A comparison of the rate of lactate accumulation during stress condition in P. indicus with values reported by other workers (Prichard & Eddy, 1979; Spotts & Lutz, 1980) for other decapods is difficult, due to differences in experimental procedure and a lack of related data.

Accumulation of vast quantities of lactate in muscle and blood during and after physical exercise was well

documented in fishes (Black, 1959; Parker & Black 1959; Black et al. 1960; Leivestad et al., 1957). Fish under stress conditions are known to be capable of undergoing anaerobic metabolism, accumulating metabolic end products which are highly acidic (Kutty, 1972). Extensive studies by Black and his colleagues with several salmonid species have shown that muscle glycogen rapidly mobilized during exercise and that substantial amounts of lactate appear immediately in the muscle. (Black et al., 1959, 1960, 1961; Parker & Black, 1959). Following the cessation of exercise, lactate diffuses slowly from the muscle resulting in a prolonged elevation of the blood lactate concentration lasting more than 12 hours if exercise was severe (Black et al., 1961).

Although, in general, a tendancy to form lactate is much less in invertebrate tissues compared to vertebrates (Hammen, 1969), there is evidence that crustaceans accumulate lactate under stress conditions. Teal and Carey (1967) showed accumulation of lactate and concurrent utilization of glycogen in marsh crab Uca pugilator under conditions of hypoxia. Philips et al., (1977) showed that in various crustaceans, lactate is produced during exercise. Lactate build up as a result of exercise has been observed in the striped shore crab Pachygrapsus crassipes (Dendiger and Schatzlein, 1973). Spotts and Lutz (1980) showed a large

and rapid accumulation of lactic acid during activity stress in two commercially important shrimps, Penaeus duorarum and Macrobrachium rosenbergii. Lactic acid accumulation as a result of laboratory exposure to anoxia was examined in two species of mud dwelling shrimp, the ghost shrimp Callianassa californiensis and the mud shrimp Upogebia pugettensis.(Prichard & Eddy, 1979).

The present study also showed the same trend, a rise in haemolymph and muscle lactic acid immediately following severe muscular activity.

The blood level of lactate during and after exercise reflect muscle concentrations. Although produced in muscle tissue they seep into the haemolymph, often causing a drop in blood pH (Black, 1960). Following exercise the lactate diffuses slowly from the muscle resulting in a prolonged elevation of blood lactate concentration lasting more than 12 hours if the exercise was severe.

Lactate concentration was always higher in muscle than in the haemolymph (Philips et al. 1977). The present study showed a three fold increase in muscle lactate concentration after 30 minutes of strenuous exercise, and the level showed a further increase in 50 minutes exercised

prawns. Muscle lactate concentration declined slowly upon cessation of exercise and reached almost normal level after 24 hours of recovery.

Karlson (1965) pointed out that lactic acid is produced under stress conditions because of a switch from aerobic to less efficient anaerobic glycolsis which is required to maintain the production of ATP. An increase in the lactic acid concentration in the muscle and haemolymph after continuous stress as compared to control can be explained on the basis of glycogen metabolism. During intense muscular activity the metabolic rate also increases and may exceed the animals ability to consume oxygen. At such times the muscle tissues resort to anaerobic production of energy, accumulating metabolic end products which are often highly acidic. Although many end products of anaerobic metabolism are possible, lactic acid is the most important end product in crustaceans, as in fish and mammals.

A slow return of blood lactate concentration to resting levels after severe exercise is characteristic of many crustacean species (Philips et al., 1977). The metabolic fate of accumulated lactate in decapods is uncertain, for although Zebe (1982) found that some lactate was excreted by Upogebia pugettensis during exposure to

anoxia, there is currently no evidence to indicate that lactate is excreted by decapods during recovery period (Bridges and Brand, 1980). There is, however, some evidence that lactate is converted into glucose, since gluconeogenesis from lactate has been demonstrated in the Australian yabbie, Cherax destructor (Philips et al., 1977).

Although a number of studies have been directed towards studying the occurrence and distribution of the various classes of lipid substances in crustacea, knowledge of their lipid distribution until recently was limited.

Though the lipids are powerful sources of energy, they are not utilized for the production of energy for immediate muscle contraction, mainly because of the fact that metabolism of lipids is slower when compared to carbohydrates.

Renaud (1949) measured the changes in carbohydrates, lipids and protein of Cancer pagurus under a variety of conditions. The results of his work led Vonk (1960) to conclude that crustacean metabolism is mainly centered around glycogen and fatty acids; but neither glycogen (Neiland and Scheer, 1953) nor lipid levels (Monn, 1963) appear to be sensitive to starvation over periods of several weeks. There is however evidence that fatty acids are readily available (oxidized) to respiratory carbondioxide or incorporated into lipid substances by crustaceans.

The effect of stress on lipid metabolism is far from clear. Recently more attention has been paid to lipid metabolism in fish. This entailed research on the effect of stress on the free fatty acid in the blood plasma. Studies on rainbow trout, Salmo gairdneri showed that there is slight increase in free fatty acid concentration immediately after 20 minutes of hypoxia (Mazeaud et al., 1977). In another fresh water teleost, the carp, two hours of hypoxia led to a pronounced decrease in free fatty acids. The same animals, when they were submitted to forced swimming until exhaustion occurred, showed a similar decrease of free fatty acids. The reason for these results is not yet known (Mazecurd et al., 1977).

The present investigation showed a decrease in total lipid level in both muscle and haemolymph, immediately after 30 and 50 minutes of exercise. It may be that prawns utilize lipids for the energy production when their carbohydrate resources are used up. Since there are no other studies relating stress and lipid level it is premature to say that lipids are utilized for the production of energy, during strenuous activity.

Proteins are relatively abundant consistuents of crustaceans. The catabolism of proteins and amino acids

can serve as a significant source of metabolic energy since they are the major constituents of crustacean tissue. At present there is no clear evidence to decide whether the basic energy requirements of crustaceans were met predominantly by either carbohydrates, protein or lipid. Since the metabolism of protein and lipid are relatively slow, they usually do not serve as the immediate source of energy for muscular activity.

Studies conducted on the effect of starvation on protein metabolism confirmed that proteins are catabolised to meet the organisms need. Starvation studies on cray fish O. limosus reduced total body protein by only 2% after 15 days and by 11% after 41 days.

Studies on fishes showed that nitrogen metabolism is more intensive during forced activity. The total protein in the serum increases (Kondrat'eva, 1978) in Trachurus trachurus, while free amino acids are consumed and decreased significantly (Mehrle et al., 1971).

The results of the present study did not show any significant change in the total protein content of haemolymph and muscle. Protein content before and after exercise remained almost same. Statistical analysis also proved that there is no significant difference between normal, stressed, and stress recovered prawns.

In the present study, muscle opacity was observed in the abdominal segments of strenuously exercised prawns. Among penaeid prawns this muscle opacity is variously known as spontaneous muscle necrosis, muscle necrosis, ideopathic muscle necrosis, etc. Earlier studies showed that muscle necrosis is related to environmental stressors including extremes and sudden fluctuations in salinity, temperature, dissolved oxygen, overcrowding, physical handling, hyperactivity, exposure to air etc. (Rigdon & Baxter, 1970; Sindermann, 1977; Lakshmi et al., 1978; Lightner, 1983). Such a stress related muscle necrosis has been reported earlier in penaeids (Rigdon & Baxter, 1970; Venkataramiah 1971; Lightner 1983; Lakshmi et al., 1978) and Macrobrachium rosenbergii (Nash et al., 1986). Hyperactivity during intense exercise may be the major cause of opaque white discolouration of the abdominal muscle found in P. indicus. They found that this condition is reversible in its earlier stages if the adverse environmental stressors are removed, otherwise will lead to death in later stages (Lightner, 1983).

The morphological and histological changes in muscle observed in the present study, after strenuous physical exercise were similar to those previously described in penaeid and non penaeid prawns (Rigdon & Baxter 1970; Lakshmi et al., 1978). Rigdon & Baxter, (1970) Lakshmi et al.,

(1978) observed muscle opacity in the distal abdominal segments of the prawn, but muscle opaqueness was observed in all the abdominal segments as patches in P. indicus. Histopathological observation of the necrotic foci revealed extensive myofibre degeneration typical of necrotic tissue. Muscle cells in the necrotic zone exhibited varying degrees of structural degradation manifested as disorganised myofibrils and loss of recognizable sarcomeres. Muscle cells appear swollen at the necrotic foci.

Biochemical estimations carried out in the present study showed a large and rapid accumulation of lactic acid in the muscles of strenuously exercised prawns. Muscles also showed a rapid decrease in glycogen content. Intense muscular activity during strenuous physical exercise is usually followed by a period of reduced activity leading to complete exhaustion and immobility. This state has been correlated with lactic acid accumulation (Spotts & Lutz, 1980), and with the occurrence of muscle necrosis in prawns (Nash et al., 1986). Lactic acid is believed to be the major cause of post activity acidosis in crustaceans (Philips et al., 1977) and is shown to accumulate in more than 6-fold in hyperactive Macrobrachium rosenbergii (Spotts & Lutz 1980). It is shown that stress induced hyperactivity always leads to muscle hypoxia; this and

the accumulation of lactic acid during anaerobic glycolysis were the most important steps in the manifestation of muscle necrosis. During intensive muscular activity, the muscles rapidly consume the glycogen reserves generating local heat and eventually lactic acid, both of which induce degenerative changes in these and adjacent muscle fibres of all types (Hulland, 1955).

Information is generally meagre regarding histological changes observed in the hepatopancreas of strenuously exercised prawns. Nash et al. (1986) observed prawns with muscle necrosis additionally displayed a paucity of normal cytoplasmic fat/glycogen vacuolation of the hepatopancreatic epithelial cells. Besides these, some of the cells also showed an increased basophilia, rounding up and luminal sloughing which led to a focal to multifocal tubular disorganisation. Observation of the frozen sections of hepatopancreas of normal and stressed prawn stained with oil red showed that the distribution of lipid droplets are almost similar in both cases. Excessive vacuolation observed in haematoxylin and eosin stained sections of hepatopancreas might be the result of excessive accumulation of water due to increased osmotic disturbances during stressed condition. This condition of accumulation of water in the cells is known as hydropic degeneration (Jones & Hint, 1983).

The cause of this phenomenon is the failure of ionic pump mechanism. Cells are not in equilibrium with their environments and an energy dependent active process(ionic pump) is required to counteract the leaking of sodium and water into the cell and potassium from the cell through the permeable cell membrane. Any failure in the pump results in an influx of cations and water in an attempt to reach equilibrium with environment (Jones & Hint, 1983).

SUMMARY

1. The objective of the present study was to evaluate the effect of continuous physical exercise of shorter period on the biochemical constituents of muscle and haemolymph of the prawn Penaeus indicus, and also the associated histological changes in the muscle and hepatopancreas.

2. Biochemical parameters studied were glucose, lactic acid, lipid, and protein in haemolymph and glycogen, lactic acid, lipid and protein in muscle respectively. Histological observations of the muscle and hepatopancreas of normal and stressed prawns were also made to observe the changes occurring at cellular level.

3. Glucose content in the haemolymph showed a rapid increase after 30 and 50 minutes of strenuous exercise. The value came down to almost normal level after 24 hours of recovery.

4. Haemolymph lactic acid also showed a similar trend to that of haemolymph glucose. After 30 and 50 minutes strenuous activity the lactate level showed a steady rise from the normal value. After 24 hours of recovery the lactate level came down to near normal value.

5. Lipid level in haemolymph showed a slight decrease from normal level after 30 and 50 minutes strenuous exercise. The stress recovered prawns showed a lipid level similar to that of normal prawns.

6. Haemolymph protein content did not show any significant variation among stressed and normal prawns. The value remained almost same in all the four phases.

7. Muscle glycogen showed a drastic reduction immediately after stress conditions. After 30 minutes of strenuous exercise the glycogen value became less than half of t the normal level. The level showed a further decrease after 50 minutes exercise. 24 hours of recovery did not show a significant change in glycogen level.

8. Lactate content in the muscle showed a similar pattern of change as in the case of haemolymph lactate. 30 minutes of strenuous exercise showed a large and rapid accumulation of lactic acid. The level showed a further increase after 50 minutes exercise and almost became normal after 24 hours of recovery.

9. Lipid content of the muscle showed a slight decrease after 30 and 50 minutes of severe exercise. The stress recovered prawns showed a near normal value of muscle lipid.

10. Protein content in the muscle remained the same in both normal and stressed prawn. No significant variation was observed in the muscle protein content of normal, stressed, and stress recovered prawns.

Histological observations were made on hepatopancreas and muscle tissue of normal and stressed prawns.

Histological observation of the hepatopancreatic tubules of stressed prawn revealed an extensive vacuolation, when compared to normal, may be because of the accumulation of water due to osmotic disturbances, during stress conditions. Histology of opaque muscle showed marked morphological and cellular changes characteristic of progressive segmental myofibre degeneration.

The earliest changes observed in the opaque muscle swelling and loss of usual cross striations. Pyknotic nuclei are often found in the muscle cells. Haemocytes were frequently observed infiltrating the foci and areas of myofibre necrosis. Opaque muscles also showed large, round or elongated vesicular sarcoplasmic nuclei with prominent nucleoli and clumped pyknotic nuclei.

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