CHAPTER 5

EFFECT OF EXERCISE ON SUCCINATE DEHYDROGENASE (SDH) ACTIVITY AND GLYCOGEN CONTENT IN THE RAT DIAPHRAGM

The diaphragm is a mixed muscle consisting of three types of muscle fibres. These fibre types can be easily distinguished after staining for the histochemical demonstration of SDH as well as fat. They include narrow red fat loaded fibres showing the highest SDH activity; white broad glycogen loaded fibres showing no SDH activity and the intermediate fibres which show lower SDH activity as compared to the red fibres. In the rat diaphragm also these three types of fibres can be easily distinguished.

With respect to metabolism and working, the mammalian diaphragm occupies an intermediate position between occasionally contracting skeletal muscle and constantly working heart muscle. It starts its activity with the first breath of the mammal and stops with the death. For this type of sustained activity it also utilizes fat (Susheela, 1963) as the chief fuel as in the flight muscles of pigeon and bat. For the oxidation of fat the required oxidative enzyme like SDH is also present in the considerable concentration.

Various studies have been conducted in order to study the changes in level of metabolites and the enzymes under different conditions like starvation and strenuous exercise. The effect of starvation on the fat, protein, SDH and lipase in the rat diaphragm were studied by George and Susheela (1961). The present studies were conducted in order to study the changes in the level of SDH activity and the glycogen level during the different durations of exercise. The swimming exercise was selected and the temperature in the water bath is kept constant in order to prevent the change in the level of SDH and glycogen content due to the variation in the temperature of the surroundings. The effect of muscular activity of various duration on the oxidised and reduced nicotinamide adenine dinucleotide (NAD and NADH) in the muscles, liver and blood was studied by Fedorova (1964). To study the above effect the rats were given the swimming exercise. The factor of the temperature should be considered because of the fact that the rate of oxygen consumption (maximum metabolism) was highest at the lowest ambient temperature and lowest at the highest ambient temperature (Herreid, 1963). This means that, at a lower temperature the energy requirement of the animal body is higher in order to raise the body temperature.

As the diaphragm is not the homogeneous structure but consisting of the distinct regions as described by

George and Susheela (1961, 1963), all the regions were considered as separate regions in the present work.

MATERIALS AND METHODS

Freshly trapped wild rats were used in the present investigation. Some rats were dropped in the water bath for finding out the period for which they can normally swim. The rats which were not given any swimming exercise were taken for the control experiments. The rats were given exercise for different periods in the bath tub; like 15 minutes, 20 minutes, 30 minutes, 45 minutes and one hour. The diaphragm was immediately collected after decapitating them, it was then spread on the clean filter paper and blotted well to remove blood. The three regions were separated as described in the previous chapters. The quantitative study of the SDH activity and the glycogen content were made in each of the three regions. The SDH activity in a sample was determined by the colorimetric method of Kun and Abood (1949) using 2:3:5-Triphenyl - Tetrazolium chloride (TTC). The incubation mixture contained 0.5 ml. of 0.1M phosphate buffer of pH 7.4, 0.5 ml. of 0.2M Sodium succinate 1.0 ml. of freshly prepared 0.1% TTC solution and 1.0 ml. of tissue homogenate. After shaking the tubes were kept in a water bath for incubation at 37°C for 30 minutes. The enzyme activity was stopped by an addition of

7.0 ml. of acetone. The tubes were then centrifuged for 5 minutes at 3000 r.p.m. The intensity of the colour of the clear supernatant was measured on a Klett-Summerson colorimeter using 420 mμ filter. The enzyme activity is expressed as μg formazan formed per mg. protein per 30 minutes. The protein in the same samples was estimated by using the biuret reagent. The homogenate prepared for SDH estimation was diluted by taking 1.0 ml. of the homogenate and diluting it to 10.0 ml. by adding distilled water. To 1.0 ml. of the diluted homogenate 4.0 ml. of the biuret reagent was added. The tubes were shaken well and kept for 30 minutes at room temperature. The intensity of the colour was estimated on a Klett-Summerson colorimeter using 540 mμ filter.

The glycogen in the samples were estimated by using the anthrone reagent (Scifter, et al., 1950). The quantity of glycogen is expressed in terms of percentage in wet tissue or gm. glycogen per 100 gms. of wet tissue.

The tissue obtained from a single rat was found to be insufficient and so tissue taken from a number of individuals, irrespective of sex was pooled together for SDH estimation.

For glycogen estimation the tissue was collected from a single rat only.

OBSERVATIONS AND DISCUSSION

The results obtained are presented in Table 1 and graphs are also plotted in order to show the changes in the level of SDH activity and the glycogen content because of swimming exercise (Graph No. 1 SDH; Graph No.2 Glycogen content).

The three regions differ not only morphologically but also physiologically. The R.Q. (respiratory quotient) of different regions of rat has been studied by Susheela and George (1963). They have shown that R.Q. of dorsal region is 0.7, that of lateral region is 1.0 and that of the ventral region is 0.85. The above observations made by them led to the conclusion that the dorsal region utilizes fat, the ventral also utilizes fat but to a lesser extent while the lateral region makes use of the carbohydrates as metabolites. Amongst the three regions the lateral region is the active region, taking an active part in the respiratory movement, well adapted for carbohydrate metabolism. The study of the blood supply to the rat diaphragm has also revealed the fact that the lateral region has the major blood supply, while the ventral has the least amongst the three regions (Beck and Baxter,1960). The study of cholinesterases also made it clear that the lateral region of the diaphragm is the most active region,

Table 1

The effect of swimming exercise on <u>SDH</u> and <u>Glycogen</u> level in the different regions of Rat Diaphragm

Period of Exercise		Normal (no exercise)	15 mins.	20 mins.	30 mins.	45 mins.	1 hour
Dorsal Region							
SDH	Mean	2.6804	3.2002	4.3960	3.4840	3.2763	3.4780
	S.D.	±0.6498	±0.5878	±0.8109	±0.5895	±0.7972	±0.7211
GLYCOGEN	Mean	0.2478	0.6869	0.4581	0.1674	–	–
	S.D.	±0.0404	±0.0204	±0.1227	±0.0691	–	–
Lateral Region							
SDH	Mean	4.2202	3.7374	4.0090	3.0000	3.8136	4.2290
	S.D.	±0.5548	±0.8893	±0.9557	±0.7561	±0.5331	±0.8754
GLYCOGEN	Mean	0.2436	0.3860	0.3856	0.1863	–	–
	S.D.	±0.0483	±0.0141	±0.0225	±0.0855	–	–
Ventral Region							
SDH	Mean	2.0640	2.0880	1.9500	1.5700	2.0352	2.3550
	S.D.	±0.4629	±0.4452	±0.5122	±0.3572	±0.3653	±0.4152
GLYCOGEN	Mean	0.5148	0.3785	0.3667	0.1473	–	–
	S.D.	±0.0506	±0.0957	±0.0949	±0.0162	–	–

Note: SDH – The quantity of SDH is expressed in terms of µg. Formazan/mg. protein/30 minutes.
GLYCOGEN– The quantity of glycogen is expressed in terms of % or Gms. Glycogen/100 gms. of wet tissue.

because of the presence of high concentration of cholinesterases in the lateral region particularly the acetyl cholinesterase.

When the rats are given the swimming exercise it can be clearly observed that the oxygen requirement of these rats increases. To increase the intake of oxygen the respiratory rate also increases, because of which the activity of diaphragm also increases. Nova _et al_. (1962) worked on the ventilation, oxygen consumption and utilization in lungs by the adult boys during physical exercise. This investigation of the respiratory function was carried out in 13-18 years old boys on a bicycle ergometer. They found that all boys reacted equally to the greater exertions with equal minimum oxygen consumption, but the oxygen consumption rose in proportion to increasing exertion. It was also observed by the above authors that the more developed boys require more oxygen than the boys with poor physical development.

From the results recorded in the Table 1 and the Graphs 1 and 2 it can be clearly seen that the glycogen level in the dorsal and the lateral region increases within the first 15 minutes. The increase is again more after 20 minutes of swimming exercise. After 20 minutes the level again falls down as it is observed at the end of 30 minutes.

The glycogen level in the ventral region falls down gradually as it appears from the observations at 15, 20 and 30 minutes.

The study of glycogen level shows that the diaphragm must be utilizing glycogen for a short period to meet the increased need of energy, but it can not work continuously with its glycogen reserves. Hence the glycogen level falls down after 30 minutes. The rise in glycogen level at the end of 15 and 20 minutes indicates that the diaphragm is utilizing more of glycogen for which the substance is either brought there in the organ by the blood or the muscle fibres must be synthesising it. The recent studies by Stubbs and Blanchaer (1964) on glycogen, phosphorylase and glycogen synthetase activity in the red and white muscle fibres of guinea pig has shown that because of stimulation the glycogen synthetase activity of the red fibre increases and more glycogen is synthesised, which proves that the increase in the glycogen level must be due to the synthetic activity of the red fibres of the diaphragm.

The study of SDH activity shows that in the dorsal region the enzyme activity gradually increases during the short period of 15 to 20 minutes. After 20 minutes the level again falls down and remains constant at 45 minutes and 1 hour. Still the enzyme activity is found to be higher than was observed in the controlled specimens. From these observations it can be concluded that the enzyme activity increases upto 20 minutes in order to meet with the increased need as was observed in the quantitative study of glycogen. The lateral region also gave similar results with slight difference. The lateral region initially shows high enzyme activity which is lowered down at the end of 15 minutes. At 20 minutes it again increases and then reduces at 30 minutes of exercise. From 30 minutes to 1 hour it gradually increases, and at the end of 1 hour it attains the same level as the rats which were not given any exercise. This shows that the lateral region maintains its high level of enzyme activity because it is an important and active region of the diaphragm. Thus it can also be proved that the lateral region is capable of oxidizing both carbohydrates as well as fats. The ventral region showed the least activity of SDH at the initial stage which has slightly increased at the end of 15 minutes, from 20 to 30 minutes it gradually decreases. At 45 minutes of exercise a rise in the level of SDH activity is noted again and at the end of 1 hour it attains the same level as the unexercised rats.

In all the three regions the level of SDH activity after one hour attains the same level as the control experiments, but slight increase is still observed which can be due to the extra work load (the oxygen requirement for swimming in addition to the normal activity) which the diaphragm has to carry out.

The study of the change in the glycogen level and SDH level clearly reveals two facts. One is that the organ is synthesising more of glycogen in the early period of exercise because there are many red fibres (red fibres are more than the white and the intermediate fibres Chapter 1). Second is that the organ uses fat for long, sustained and strenuous activity with the help of oxidative enzyme.

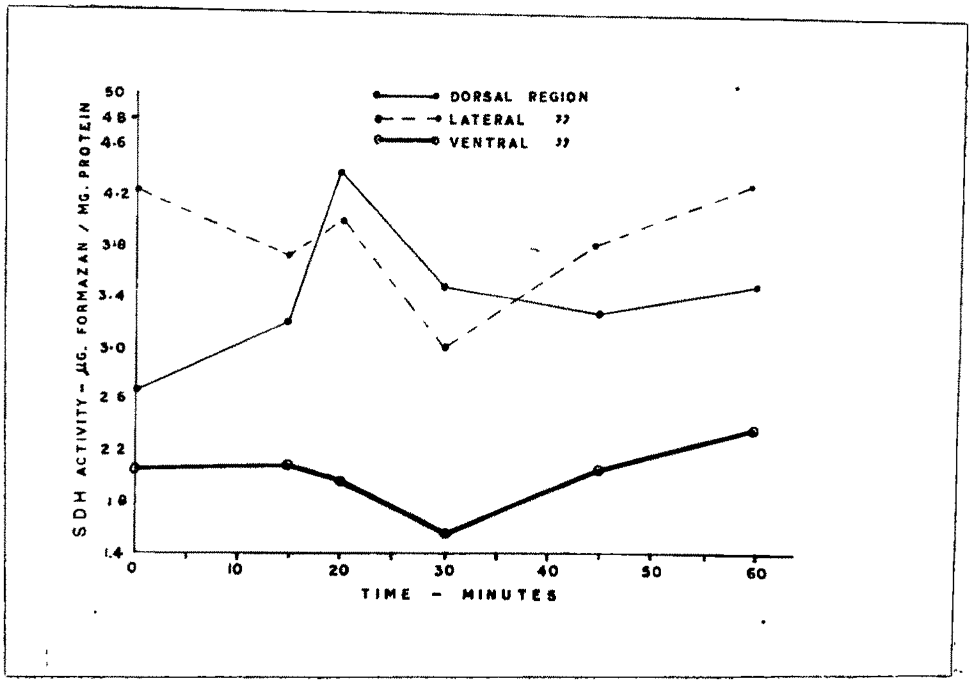

Fig.1: A graph showing the effect of exercise on the SDH (Succinate dehydrogenase) activity of rat diaphragm. The activity of the enzyme is expressed in ug. Formazan/mg. Protein/30 minutes at 37°C. Time-minutes indicates the period of exercise.

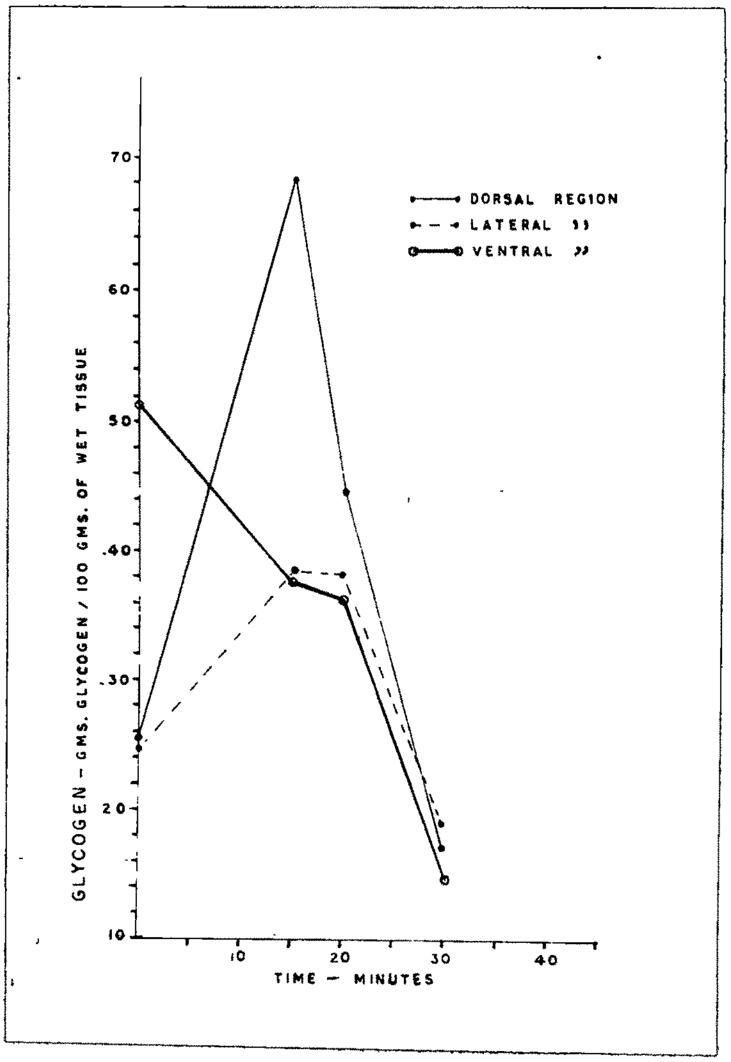

Fig. 2: A graph showing the effect of exercise on the Glycogen level of rat diaphragm. The glycogen level expressed in terms of Gms.Glycogen/100 Gms. of wet tissue. Time-Minutes indicates the period of exercise.