CHAPTER 2

LIPASE ACTIVITY IN THE VERTEBRATE HEART MUSCLE AND ITS RELATION TO BASAL METABOLISM

Most of the mechanical energy involved in heart beat is used in expelling blood into the arteries against pressure and the minute volume of circulation or the cardiac output per minute is dependent on the pulse rate and stroke volume (Dukes,1947). The pulse rate of different animals vary according to size and age. The cardiac out put which is calculated by multiplying the stroke volume by the pulse rate is greater per unit weight of the body in small animals. This is indicative of the fact that smaller animals have a higher metabolic rate per unit body weight (Dukes,1947). Brody (1945) pointed out that as a rule the pulse rate is directly proportional to the basal oxygen consumption per unit body weight in mature animals of different species and is inversely proportional to body size. But Prosser (1950) considers it probable that activity is more important. Variations in heart beat of animals are influenced by the various conditions that affect the basal metabolism of animals in general such as season, age, sex, activity, environmental temperature,pathological conditions etc.

In heart muscle therefore there should be a tremendous expenditure of energy depending on the heart rate. Contrary to the classical belief that carbohydrates form the chief energy source in the heart muscle, the recent work of

Bing and his collaborators (Bing, 1954) of sampling the blood drawn from the coronary sinus by the catheter technique, the most reliable method available at present, for studying the metabolism of the heart, has shown that nearly 67% of the required energy in humans is derived from fatty acids and only a portion of the remaining from carbohydrates. It has been observed in birds and in certain other flying animals like bats and locusts that fat is the main fuel during long and sustained muscular activity (George and Jyoti, 1955a and '57, Weis-Fogh, 1952). My finding that there is a lipase in the vertebrate skeletal muscle and that there is a quantitative variation depending on activity in different muscles of the same animal and the same muscle of different animals supports this view. It was also suggested that the lipase concentration of a muscle would depend on the extent of fat utilization, which in turn could be correlated with the nature of activity of the muscle. Therefore it could be expected that the heart muscle which performs sustained activity would show a very high lipase activity and a quantitative difference in different animals depending on the metabolism of the animal. This study was thus undertaken to see if some experimental evidence could be made available in order to substantiate these assumptions.

Material and Methods

The lipase activity of the heart muscle of the following animals was assessed.

Frog (*Rana tigrina*)	Freshly collected
Turtle (*Lissemys punctata*)	Laboratory animal
Pigeon (*Columba livia*)	Wild
Parakeet (*Psittacula kramari*)	Wild
Kite (*Milvus migrans*)	Wild
Eagle(*Aquila rapax*)	wild
Sparrow (*Passer domesticus*)	Wild
Fowl (*Gallus domesticus*)	Domestic
Bat (*Hipposideros speoris*)	Wild
Rat (*Rattus rattus*)	Wild
Rabbit (*Oryctolagus cuniculus*)	Laboratory animal
Sheep (*Ovis aries*)	Slaughter house material, collected immediately after decapitation
Horse (*Equus caballus*)	Heart cut out about an hr. after death

Enzyme Material

A glycerine extract of the heart muscle was prepared as described in the last chapter. In the case of small animals like sparrow, rat, bat and parakeet a number of them irrespective of age and sex were sacrificed for each set of experiments to procure the required quantity of the muscle. The blood was completely removed with filter paper and only the muscle of the ventricle used for the assay.

Method

The method of estimation was essentially the same as used in chapter I, but in many cases the quantity of the reaction mixture was reduced to one half and contained 5 ml. of the emulsion, 2.5 ml. buffer and 2.5 ml. enzyme preparation,

thus making up a total volume of 10 ml. and correspondingly the quantity of the alcohol-acetone mixture used to stop the reaction was also reduced to one half. The pH was adjusted to 8 and the reaction mixture was incubated for 4 hrs. at 40°C.

Calculation

The lipase activity is referred to as the lipase value which is expressed as the number of lipase units per gm. of the wet muscle and was calculated according to the definition (Chapter 1)

Results

The values obtained for the heart of different animals is given against the heart rate and/or the metabolic rate in the respective animals taken from available previous literature (Table 1)

Table 1

Data on the relation between the metabolic rate and the lipase value of the heart muscle of various animals

Animal	Heart rate (Author in parentheses)	Metabolic rate		Lipase value
		Oxy.conc.cc./ gm./hr.(Author Heilbrunn)	Heat prod.24hrs./ kg.body weight (Author-Brody)	
Frog		0.21		6.4
Turtle				3.3
Pigeon	185(Heilbrunn)		102	11.2
Parakeet	320(Heilbrunn)		227	10.6
Kite				11.1
Eagle				10.4
Sparrow	800(Heilbrunn)	6.71	231	26.7
Fowl	150-180(Prosser)	0.83	52	9.4
Bat				15.2
Rat	300-500(Prosser)	0.692	83	22.2
Rabbit		0.64	45	27.7
Sheep	70-80 (Dukes)	0.34	26	6.7
Horse	32-44 (Dukes)	0.25	17	3.0

Lipase Value in Relation to Age and Sex

The animals chosen for this study were rats and sparrows, the sparrows for sex differences and the rats for sex as well as age. The weight of the animals was determined and used as an index of age. Thus the rats were classified into the following three weight groups.

A Above 100 gm.

B 50 - 100 gm.

C Below 50 gm.

In the first two groups the males and females were separated and the lipase value of the heart assessed separately. A number of animals were sacrificed for each experiment and the values given below are the average of three experiments each one done in duplicate.

Table 2

Showing the lipase value of the heart muscle of rat and sparrow in relation to age and sex

Animal	Group	Lipase value	
		Male	Female
Sparrow		24.0	22.0
Rat	A	26.4	17.6
Rat	B	28.2	23.0
Rat	C	18.5	

Discussion

According to Kleiber (1932), the influence of body size on metabolism may reasonably be related to oxygen transport. Henderson's (1923) results demonstrated a direct

proportionality between circulation rate and pulse rate and it has been suggested that pulse rate be used for determining metabolic rate. So if the size of the heart were directly and the pulse rate inversely proportional to the body weight, in small animals, per unit weight of the body, the heart muscle should expend a greater amount of energy than in larger animals. It has been shown that the heart muscle is equipped with all the enzymes necessary for the oxidation of fatty acids (Green) 1954). If fat is the major source of energy for the activity of the heart, the heart muscle of smaller animals should have a better equipment of the enzyme systems responsible for the degradation of fat into the final products of oxidation. The concentration of lipase in the heart muscle is important because this enzyme is concerned with the breakdown of fat into fatty acids and glycerol. Table 1 shows that there is a difference in the lipase value of the heart in different animals and it roughly conforms to the difference in the metabolic rate. A similar difference may also be expected in the case of the other enzymes of the " fatty acid cycle ". Since the values presented in Table 1 cannot be considered as absolute, it is not my intention to formulate any mathematical relationship between metabolism and lipase value. It only shows that there is a significant relationship between the lipase value and the metabolic rate and supports the assumption that mostly fat is utilized for energy purposes in the heart and the contention that the lipase concentration of a muscle would depend on its activity and consequently the utilization of fat in the muscle

Table 2 shows the lipase activity of the heart muscle of rat in relation to age and sex and of sparrow in relation to sex. In both cases there is an appreciable difference in the lipase value of the male and female, the male having a higher value and it is known that the metabolic rate of the male is higher than that of the female. Among the three age groups in rats, the highest value is obtained for the B group . This is in conformity with the age curves of metabolism in rats. According to the figures of Brody (1945) the metabolism per unit surface area rises and in the case of rats it rises from about 400 cal./sqm./day near birth to 1100 - 1200 at the age of 40 days or body weight of 100 gm. Thereafter the metabolism declines to about 800 cal./sqm./day at the age of few months or weight of 300 gm. It might therefore be concluded that the mechanism behind these metabolic changes is enzymic and shows the ability of the animal to derive energy by metabolizing high energy metabolites by the development of the appropriate enzyme systems.