CHAPTER 7

OXYGEN CONSUMPTION AND OXIDATION OF VARIOUS METABOLITES BY THE PIGEON ADIPOSE TISSUE, *in vitro*

Adipose tissue has long been regarded as a storage form of connective tissue with very little metabolic activity of its own. However, it is being realised more and more at present, that this belief is illfounded. Sufficient evidence has already accumulated to show that it is metabolically active and is even on par with other well-known metabolically active tissues. The *in vitro* oxygen consumption by the adipose tissue of the rat was studied by Mirski (1942) and of the ground squirrel by Hook and Barron (1941). But so far as I am aware, there is no comparable work on the avian adipose tissue. In the present study, the oxygen consumption by the pigeon adipose tissue (in Ringer phosphate and Krebs-Ringer bicarbonate media, without added substrates and co-factors), and the oxidation of various metabolites by the same tissue in the absence of co-factors, have been studied, *in vitro*. The *in vitro* oxygen consumption by the brown and yellow adipose tissue of a flying mammal, the bat (in Ringer phosphate medium), was also studied for comparison.

Material and Methods

The material used was the visceral adipose tissue, covering the coils of the intestine, of the pigeon (*Columba livia*). After decapitation of the pigeon the tissue was taken and transferred to a cold watch glass placed on cracked ice and was then cut into small slices with a pair of clean scissors. The

subcutaneous, interscapular brown adipose tissue and yellow adipose tissue from the lower lateral sides of the abdomen, were carefully removed after decapitation of the bat (Taphozous sp.), transferred to separate, cold watch glasses placed on cracked ice and was sliced as before.

The oxygen consumption by the adipose tissue in the absence of added substrates and co-factors was studied as follows. 100 ml of 0.154 M NaCl, 4 ml of 0.154 M KCl, 1 ml of 0.319 M $MgSO_4$ and 30 ml of phosphate buffer of pH 7.4 were mixed and stored at 4°C. The final pH of the mixture was 7.4. 3 ml of this was taken as the incubation medium, in each Warburg flask having centre well and one side arm. In addition to the above Ringer phosphate medium, Krebs-Ringer bicarbonate medium was also tried in the case of the pigeon. This medium was made up of the following: 100 ml of 0.154 M NaCl, 21 ml of 0.154 M $NaHCO_3$, 4 ml of 0.154 M KCl, 1 ml of 0.154 M KH_2PO_4, 3 ml of 0.11 M $CaCl_2$ and 1 ml of 0.154 M $MgSO_4$ (Hagen et al,1959). In each set of experiments the adipose tissue from the same pigeon was used for both the media. This was done with a view to compare the amount of oxygen consumed in these two media by the adipose tissue slices. The flasks were kept cool till they were transferred to the bath (37°C), by keeping on cracked ice. The slices of adipose tissue were weighed rapidly and transferred into the flasks by means of a pair of cold, clean, dry forceps. 100 - 300 mg of the tissue was taken in each case. The centre well contained 0.2 ml of 10% KOH with filter paper, thus making up a total fluid volume of 3.2 ml. Incubation was carried out for 1 hour at 37°C with air as the gas phase. After

an equilibration period of 10 minutes, the readings were taken at regular intervals for 1 hour. The manometers were shaken at 90 - 96 oscillations/ minute with an amplitude of 2.5 cm per oscillation. The results are expressed as µl O_2 consumed/ mg protein/ hour and µl O_2 consumed/ 100 mg wet tissue/ hour. Protein content of the adipose tissue was determined by the micro-Kjeldahl steam distillation method for total proteins (Hawk et al,1954). For the determination of protein 20 - 40 mg fresh tissue was taken.

For the study of the oxidation of various metabolites (glucose, glycogen, sodium succinate, pyruvate, lactate, citrate, acetate, glycerophosphate), the same procedure was followed. The volume of Ringer phosphate was reduced from 3 ml to 2.5 ml and to this 0.5 ml of 0.1 M solution of the metabolite was added except in the case of glycogen and glycerophosphate, where the concentration of the solution used was 0.1% instead of 0.1 M. 0.2 ml of 10% KOH was kept in the centre well with filter paper. Control flask contained 3 ml Ringer phosphate solution in the main chamber and 0.2 ml of 10% KOH in the centre well with filter paper. Both sample and control flasks each contained in addition 100 - 300 mg of sliced tissue which was obtained from a single site. The values given for the oxidation of the various metabolites are the figures obtained after substracting the values of oxygen consumed by the tissue in the control flask from that of the sample.

In the presence of added substrates like glucose and acetate, synthesis of fatty acids may also take place. To investigate this aspect, the following procedure was adopted (Ball et al, 1959). 100 - 300 mg of adipose tissue was placed in a Warburg flask with 3 ml Ringer phosphate containing 3 mg/ ml glucose or

acetate, without any KOH in the centre well, for the absorption of CO_2, with air as the gas phase. The flasks were kept cool till they were mounted and transferred to the bath (37°C). After an equilibration period of 10 minutes, the readings were taken after 1 hour. If the oxygen consumption of the tissue equals its carbon dioxide output, then no pressure change should be observed. If on the other hand, carbon dioxide output exceeds the oxygen consumption, then a positive pressure should develop which could be measured. If the reverse is the case (R.Q less than 1), then a negative pressure should develop which could be also recorded. Ball et al (1959) applied this method to a study of fat synthesis by the adipose tissue of the rat.

Results and Discussion

Table 1 presents values of the oxygen consumption of the adipose tissue, in the absence of added substrates and co-factors, in the two media, Ringer phosphate and Krebs-Ringer bicarbonate. From the results obtained it is evident that Ringer phosphate is a better medium than Krebs-Ringer bicarbonate. According to Hagen et al (1959) Krebs-Ringer bicarbonate medium is the most satisfactory of all the media tried by them, for the rat adipose tissue.

Table 1

Table 1

The *in vitro* oxygen consumption by the pigeon adipose tissue

Experiment	Medium			
	Ringer phosphate		Krebs-Ringer bicarbonate	
	µl O_2/ 100 mg tissue/ hour	µl O_2/ mg protein/ hour	µl O_2/ 100 mg tissue/ hour	µl O_2/ mg protein/ hour
1	11.64	4.70	12.56	5.07
2	11.79	4.76	8.53	3.44
3	8.41	3.39	5.67	2.29
4	7.57	3.06	4.98	2.01
5	13.56	5.47	12.13	4.90
6	13.68	5.52	-	-
7	20.53	8.29	-	-
Mean	12.45	5.03	8.77	3.54

Table 2 presents the values of oxygen consumption by the two types of adipose tissue of the bat. From the results presented below it is evident that oxygen consumption by the brown adipose tissue is many times higher than that of the yellow variety, when calculated on the wet weight basis.

Table 2

The *in vitro* oxygen consumption by the brown and yellow adipose tissue of the bat

Tissue *	µl O_2/ 100 mg tissue/ hour	µl O_2/ mg protein/ hour
Brown adipose	78.30	11.69
Yellow adipose	7.27	8.99

* The values presented are the average of 5 experiments.

However, this difference in activity becomes much less when calculated on the basis of the protein content. This is mainly, if not solely, due to the varying protein contents of these two types of adipose tissue. The average, total protein content of the brown adipose tissue is 6.70 mg/ 100 mg fresh tissue and that of the yellow adipose tissue, 0.808 mg/ 100 mg fresh tissue.

Of the various metabolites added, succinate was found to be oxidized to the maximum and the oxidation of pyruvate was to the minimum. Citrate also gave a very low value (Table 3).

Table 3

Table 3

Oxidation of the various metabolites by the pigeon adipose tissue

Substance *	µl O_2/ 200 mg tissue/ hour		
	Sample	Control	Difference
glucose	13.58	9.86	3.72
glycogen	20.15	14.58	5.57
succinate	36.18	11.14	25.04
pyruvate	12.58	10.83	1.75
citrate	7.75	5.89	1.86
acetate	11.08	7.65	3.43
glycero-phosphate	22.00	15.42	6.58
lactate	14.12	7.76	6.36

* The values presented in each instance is the average of 5 experiments

Mirski (1942) reported that, addition of pyruvate and glucose increased the oxygen uptake of the adipose tissue of the rat, while lactate and glycogen had no effect. In the present studies however, all the metabolites tried increased the oxygen consumption to a lesser or greater extent.

The presence of glucose or acetate did not bring about fatty acid synthesis as was seen in the experiments conducted to assess the possibility of fatty acid synthesis. The pressure inside the manometers remained slightly negative indicating oxygen consumption. The adipose tissue in such cases maintains

a R.Q below 1. Ball et al (1959) also found that fatty acid synthesis did not take place in the presence of glucose alone. When insulin was also present in the medium, fatty acid synthesis occurred (Ball et al,1959).

The protein content of the fresh adipose tissue of the pigeon ranged from 1.446 mg/ 100 mg tissue to 4.666 mg/ 100 mg tissue, with an average of 2.478 mg/ 100 mg tissue (Table 4).

Table 4

The protein content of the pigeon adipose tissue

Experiment	mg protein/ 100 mg tissue
1	3.243
2	3.115
3	1.816
4	1.577
5	2.326
6	1.803
7	1.953
8	1.792
9	2.253
10	1.446
11	4.666
12	3.745
Mean	2.478

The wide variation in the protein content of the adipose tissue is mainly due to the unequal amounts of fat present in this tissue in the different pigeons.