CHAPTER - II.

Studies on Respiration and Glycolysis of Different Normal and Tumour Tissues.

For any significance to be attached to the high anaerobic and aerobic glycolysis of tumours, similar rates of glycolysis must be shown not to be common properties of normal tissues.

Victor and Potter (1938) studied the lymph nodes from normal mice and mice with spontaneous and transmitted lymphatic leukaemia and observed that a quantitatively small but consistent increase in the anaerobic and aerobic glycolysis of the lymph nodes of the leukaemic mice.

With this impression, studies on the respiration and aerobic and anaerobic glycolysis of some normal, two types of solid tumours (namely Mouse Fibrosarcoma and Schwartz leukaemic tumour) and Ehrlich ascites cells were taken.

Materials and Methods :

1. Mouse Fibrosarcoma :- MFS tumour tissue obtained from ICRC, Bombay was maintained in our laboratory by series of transplantation and tissue homogenate was prepared as follows: 12 days old MFS tumour was isolated and cut into small pieces. Necrotic and haemorrhagic parts were rejected. The material was then chilled and homogenized in glass homogenizer (Potter-Elvehjem) in 0.154 M KCl and kept in ice bath. The homogenate was then centrifuged at 1000xg for 10 minutes in a refrigerated centrifuge at 2^{0}C to remove debris. The homogenate was then

diluted in 0.154 M KCl to a concentration of 2 gm in 10 ml. To each healthy male mouse (15-20 gm body wt.) 1 ml of the homogenate was injected subcutaneously in one hind leg. Tumour usually developed in 4-7 days. It gradually increased in size until the animal died in about 20-25 days when necrosis usually started.

Preparation of tumour homogenate :

Swiss albino mouse bearing 9-12 days old MFS tumour was killed by spinal dislocation. Necrotic tissue was discarded. A 1:10 (w/v) tumour tissue homogenate was prepared in 0.9% NaCl using a glass homogenizer in chilled ice.

2) Schartz leukaemic tumour :

A Schwartz leukaemic tumour was developed in healthy Swiss albino mice weighing 15-20 gms and maintained by serial transplantations as described in case of MFS tumour.

Preparation of tumour homogenate :

A 1:10 (w/v) tumour tissue homogenate was prepared as described above.

3) Ehrlich Ascites Carcinosarcoma Cells :

Ehrlich ascites carcinosarcoma bearing mice were obtained from Chittaranjan National Cancer Research Centre, Calcutta and were subsequently maintained in our laboratory by serial transplantation in C_3H mice weighing 20-25 gms.

Animals were killed by cervical dislocation and peritoneal fluid was aspirated out with the help of a sterile syringe and collected in cold sterile centrifuge tubes. The cells were harvested by centrifugation at 3000 r.p.m. for 30 seconds. The sedimented R.B.C. were taken out with the help of a sterile Pasteur pipette. The remaining fluid with the cells were then centrifuged at the same speed for 2 minutes. The supernatant fluid was discarded and the cells were resuspended uniformly in cold normal saline and recentrifuged as above. The process was repeated till the cells were completely devoid of any perceptable blood cells. The packed Ehrlich's cells were then suspended in sterile normal saline (1:10 v/v) and 0.4 ml of this suspension was injected intraperitoneally to obtain ascitic form.

The transplantation of ascitic form of this tumour was done every 7 days.

4) Normal Spleen, kidney and muscle tissues were taken and 10% homogenates in normal saline were made.

For measuring glycolysis and respiration of the above mentioned normal and tumour tissues the complete system usually

consisted of 0.1 ml of 0.01 M ATP (K-salt), 0.2 ml of 0.003 M NAD (K-salt), 0.3 ml of 0.4 M Nicotinamide, 0.15 ml of 0.5 M $KHCO_3$ (used only when glycolysis was determined in N_2), 0.3 ml of 0.1 M potassium phosphate buffer (pH 7.4), 0.15 ml of 0.04 M Fructose-1-6-PO_4 (K-salt), 0.1 ml of 0.12 M $MgCl_2$ (added last), 0.3 ml of 0.1 M glucose, 0.3 ml 10% homogenate (in case of normal and solid tumour tissue) or 0.3 ml peritoneal fluid (in case of ascites cells). 0.9% NaCl solution was added to make the final volume 3.0 ml. A filter paper soaked in 0.2 ml of 20% KOH was placed in the central cup of the Warburg flask when respiration was measured. When anaerobic glycolysis was measured, the flasks were gassed with Nitrogen and no KOH was added to the central well.

The Warburg flasks were attached to the manometers and placed in a water bath of 37°C. Glycolysis and respiration were measured manometrically (Umbreit et.al., 1959).

Table - 1.

Respiration and Glycolysis of some Normal and Tumour Tissues :

Results are expressed as µl/per mg. of dry tissue/per hour.

Tissue Homogenates	$-QO_2$	$Q^{O_2}_{CO_2}$	$Q^{N_2}_{CO_2}$
Spleen	6.3	0.2	5.8
Kidney	9.1	0.6	3.1
Muscle Fascia	1.2	0.1	0.9
Schwartz leukaemic Tumour	10.0	8.7	13.0
MFS	6.5	8.2	13.2
Ehrlich Ascites cells (7 days old)	22.0	20.5	64.0

$-QO_2$ denotes O_2 uptake in µl per mg of dry wt. of tumour/hour.

$Q^{O_2}_{CO_2}$ denotes CO_2 production ,, (in oxygen)

$Q^{N_2}_{CO_2}$ denotes ,, ,, (in nitrogen)

Results :- Oxygen consumption and both anaerobic and aerobic glycolysis of different normal tissues (Kidney, Spleen and muscle fascia) and three type of tumour tissues (MFS, Schwartz leukaemic solid tumour tissue and Ehrlich ascites cells) are shown in the table-1.

From the table it is seen that aerobic glycolysis of the normal tissues was almost a rarity; but tumour tissues showed a high rate of both aerobic and anaerobic glycolysis.