CHAPTER - III.

P A R T - I

Studies on the Respiration of Tumour Cells with Different Added Substrates.

Dickens (1941) found that tumours displayed a distinct pattern characterized by a high anaerobic glycolysis and a low rate of carbohydrate oxidation.

Bloch-Frankenthal et al (1957) showed using radioactive glucose that over a period of 4 hours the oxygen uptake and glucose oxidation remained linear despite a rapid glycolysis resulting in conversion of glucose to lactate. Thus in the latter part of the experimental period the glucose had disappeared and the tumour was oxidizing lactate.

Crabtree (1929) noted that addition of glucose to actively respiring malignant tissue produced a transitory decrease in the cellular oxygen uptake. This inhibition was termed as Crabtree effect. In 1956 Brin and McKee and Yushok (1964) showed that other hexose and hexose analogues could produce the Crabtree effect.

In the present study the endogenous respiration of MFS tumour cells and then the effect of glucose and some other added substrates were studied.

Materials and Methods :

Tumour Homogenate :- MFS tumour tissue obtained from ICRC, Bombay was maintained in our laboratory by serial transplantation and tissue homogenate was prepared as described in previous chapter. The tumour tissue (2 gm) was homogenized in a glass homogenizer (Poter-Elvehjem) with 0.9% NaCl in cold.

The final volume of the homogenate was 20 ml, so that each ml. of the homogenate contained 100 mg of tumour tissue.

Warburg flasks were arranged in the following groups :

1) 0.3 ml tumour homogenate was added.
2) 0.3 ml tumour homogenate and 0.3 ml of 0.05 M glucose were added.
3) 0.3 ml tumour homogenate and 0.3 ml of 0.05 M fructose were added.
4) 0.3 ml tumour homogenate and 0.3 ml of 0.2 M Na-Malate were added.
5) 0.3 ml tumour homogenate and 0.3 ml of 0.2 M Na-Oxalacetate were added.
6) 0.3 ml tumour homogenate and 0.3 ml of 0.2 M Na-lactate were added.
7) 0.3 ml tumour homogenate and 0.3 ml of 0.2 M Na-pyruvate were added.

In all the cases 0.3 ml of 0.1 M phosphate buffer pH 7.4 was added and the final volumes were made upto 3 ml with 0.9% NaCl.

The standard Warburg procedure (Umbreit et.al. 1959) was followed. The total volume in the vessels was 3.0 ml. and 0.2 ml of 20% KOH solution was absorbed on an accordioned strip of filter paper in the centre well). The temperature of water bath was 37°C, the gas phase being air. The equilibration time was 10 minutes before the first manometric reading was taken and oxygen consumption was measured every 15 minutes.

All chemicals used were of analytical reagent quality.

Table - 2.

Oxygen Uptake of MFS Tumour Cells with Different Added Substrates.

Results are expressed as µl of O_2 taken up per flask per hour.

Substrates	O_2 uptake
Endogenous	5.2
With Glucose	3.8
,, Fructose	4.1
,, Na-Malate	6.1
,, Na-lactate	8.1
,, Na-oxalacetate	9.6
,, Na-pyruvate	9.8

Results :

Table-2 shows the endogenous respiration of MFS cells and effect of added glucose, fructose, lactate, malate, oxalacetate and pyruvate on it. From the results it can be seen that when glucose and fructose were added to the homogenate then respiration was slightly lowered than that of endogenous, which might be due to Crabtree effect.

It is also evident that malate, lactate, oxalactate and pyruvate when added, the respiration of MFS tumour cells was accelerated by 17%, 55%, 84% and 88% respectively.

CHAPTER - III.

P A R T - II

Studies on Glycolysis of Tumour Tissues with Different Substrates.

In the neoplastic cells there are at least two major pathways of the utilization of carbohydrates - the Embden Meyerhof glycolytic pathway, which is quantitatively more important and the hexose monophosphate shunt (Wenner and Weinhouse, 1956).

Wenner et.al.(1958) studied the fate of labelled glucose in a number of normal and tumour tissues, in terms of conversion to lactate and to $C^{14}O_2$ via the two pathways. Studies of enzyme, coenzyme levels and specifically labelled glucose indicate that quantitatively the HMP shunt pathway in tumours appear to be at least as important as in the homologous normal tissues.

Weber and Cantero (1955, 1957, 1958) have studied the metabolism of tumours from the particular point of view of the fate of glucose-6-PO_4.

This compound has four metabolic pathways open to it, isomerization to fructose-6-PO_4 followed by glycolysis via the Embden Meyerhof scheme, oxidation to 6-phosphogluconate with subsequent oxidation via the monophosphate shunt,

isomerization to glucose-1-PO_4 prior to conversion to glycogen, and finally cleavage by glucose-6-phosphatase to glucose. It is therefore a critical intermediate in carbohydrate metabolism.

In the present chapter glycolysis of tumour tissue and effect of some added substrates on it were studied.

Materials and Methods :

MFS tumour was maintained through serial transplantation in our laboratory, and 10% homogenate of tumour tissue was prepared as described earlier. For the manometric measurements all the flasks contained the following : 0.3 ml of 0.1 M K-PO_4 (pH 7.4), 0.3 ml of 0.4 M Nicotinamide, 0.1 ml of 0.01 M K-ATP, 0.2 ml of 0.003 M NAD (K-salt), 0.2 ml of 0.1 M $MgCl_2$.

The chemicals used were obtained as follows :
ATP, NAD, K-Phosphate from Sigma Chemicals (U.S.A.), Nicotinamide from Nutritional Biochemicals Co. (U.S.A.) and $MgCl_2$ from E.Merck (Germany).

After adding the required solutions, flasks were arranged in following groups :- (0.3 ml of tumour homogenate was added in groups 1-5)

1) 0.3 ml of 0.1 M glucose was added.
2) 0.3 ml of 0.1 M fructose was added.
3) 0.3 ml of 0.1 M glucose and 0.1 ml of 0.15 M Pyruvate were added.

4) 0.3 ml of 0.1 M fructose and 0.1 ml of 0.15 M pyruvate were added.

5) Glucose-6-Phosphate containing 3 mg hexose was added.

6) Control (for endogenous) :- 0.3 ml tumour tissue homogenate was added.

To all the flasks 0.9% NaCl was added to make the final volume 3 ml.

The Warburg flasks were attached to the manometers and kept in water bath at 37°C. Manometric readings were taken after the temperature equilibrium was set up (Umbreit et.al., 1959).

Table - 3.

CO_2 Production of MFS Cells with Different Substrates.

Results are expressed as µl of CO_2 produced per flask per hour.

Substrates	CO_2 production.
Endogenous	6.4
With Glucose	9.2
" Fructose	7.6
" Glucose + Pyruvate	17.9
" Fructose + Pyruvate	16.2
" Glucose-6-PO_4	32.6

Results :-

The results in Table-3 show that the endogenous glycolysis of tumour cells takes place at lower level. In presence of glucose, fructose or glucose-6-PO_4 the glycolysis was increased. With addition of pyruvate along with glucose or fructose, the level of glycolysis was much higher.