D I S C U S S I O N

The admission of biochemistry into the group of the oncological sciences has provided the opportunity for professional chemists to initiate powerful diverse and above all, sustained lines of approach towards the elucidation and control of cancer. Since the advent of biochemical studies attempts had been made to differentiate cancer cells from the normal cells. But when one goes through all these literatures, one finds that in most cases these differences are qualitative rather than quantitative. However, it is seemingly characteristic of tumour tissues that they possess a relatively higher rate of lactic acid production under both aerobic and anaerobic conditions, than do many normal tissues. This implies an accumulation of lactic acid in tumours (a) because the particular system or systems concerned with its further degradation is lacking (b) because tumour possess some more highly active enzyme system which rapidly degrade glucose to this product or (c) because of unusual change in the metabolic pattern of tumours, the degradation of glucose is at some stage diverted to the formation of this product.

Attempts have been made to utilize this difference in screening chemotherapeutic agents for cancer. It has been thought that a compound which would change the glycolytic behaviour of tumour tissue might have a promise of an anti-cancer drug. With this idea in mind, the respiratory and

glycolytic behaviour of some tumour tissues have been studied and also the effect of a locally isolated new compound named Jawaharene is studied and compared with two well known anti-tumour antibiotics namely Mitomycin C and Actinomycin D.

The data presented in Chapter II shows the rate of aerobic and anaerobic glycolysis of SLT, MFS and Ehrlich ascites cells and some normal cells. It would be observed from Table 1 that the all three types of tumour cells studied show an increased rate of both aerobic and anaerobic glycolysis. The aerobic glycolysis of the tumour cells is more impressive than the anaerobic glycolysis, especially when it is compared with normal tissues in which aerobic glycolysis is practically negligible. This would further substantiate the findings of Warburg.

The data presented in Table 2 show that MFS cells have a low endogenous oxygen consumption. But with exogenous supply of malate, lactate, oxalacetate and pyruvate, the oxygen consumption of the tumour cells is increased, by 17%, 55%, 84% and 87% respectively. These data are more or less of a similar nature as reported by McKee et.al. (1966), who also obtained an increase in the respiration of Ehrlich-Lettre carcinoma cells when malate, lactate, oxalacetate or pyruvate were added to the incubation mixture. However, the stimulation of oxygen consumption by the MFS cells in presence of these suhstrates

are of a higher magnitude than those reported by McKee et.al. (1966). This could be due to the difference in the tumours used.

The presence of 5 mM glucose and fructose in the incubation mixture brings about a 25% decrease in the oxygen consumption of the MFS cells. In 1929, Crabtree noted that the addition of glucose to actively respiring malignant tissue produces a transitory decrease in the cellular oxygen uptake and this inhibition was termed as 'Crabtree effect'. Respiratory inhibition by glucose, fructose and mannose were also obtained by Brin and McKee (1956), Clowes and Keltch (1954), and Kunn et.al. (1951). So the MFS cells show the well known Crabtree effect, and thus behave like other tumour tissues. Thus the convergence theory of Greenstein holds good for this tumour also.

In Chapter III, Part-II, the glycolysis of tumour tissue with some added substrates has been studied. It is found that glucose gives some added glycolysis over the endogenous value. It might be possible that more glucose might be going through the hexose monophosphate shunt, thereby there is an increased amount of CO_2 production. Added fructose also gives increased amount of glycolysis over the endogenous value. Meyerhof and Wilson (1949a) also obtained a similar type of result. When pyruvate is added along with glucose or fructose then there is increased CO_2 production. Wenner et.al. (1958) and Wenner (1959) observed an increased amount of CO_2 production with labelled

glucose in presence of pyruvate in Ehrlich ascites cells. It may be reasonable to assume that the increased glycolysis in MFS cells in presence of pyruvate could be due to an acceleration of hexose monophosphate shunt pathway. From Table 3 it is further seen that when glucose or fructose is replaced by glucose-6-PO_4 then the CO_2 production is more than double than that obtained with glucose or fructose alone. Meyerhof and Wilson (1949b) observed that certain tumours could utilize hexose monophosphates more rapidly than glucose. As glucose-6-PO_4 is a key compound for either glycolysis or HMP to operate, the supply of it would bring about an increased CO_2 production.

In Chapter IV the effect of a locally isolated compound Jawaharene is studied on the respiration and glycolysis of Ehrlich ascites cells and compared with that of two well known antibiotics Mitomycin C and Actinomycin D. It is observed that Jawaharene inhibits both O_2 uptake and CO_2 production of the Ehrlich ascites cells. Chowdhury and Roy (1970) observed similar type of inhibitory effect of Jawaharene on the respiration of a mutant strain of <u>staphylococcus aureus</u>.

Since Jawaharene showed inhibitory effect of respiration and glycolysis, it was thought to be of interest to study its effect on tumour growth <u>in vivo</u>. From Table 6 and 7 it would be observed that Jawaharene has greater effect

on the inhibition of tumour growth when this compound is injected directly into the tumour than when injected subcutaneously. Ranadive et.al. (1966) also found that injecting Jawaharene directly into the tumours produced more pronounced effect than when it was injected somewhere else. The data presented in Table 6, shows the effect of Jawaharene injection on the animals bearing Ehrlich ascites cells in liquid form. Here also it is found that the volume of packed cells is decreased strikingly after injection of Jawaharene.

Coles et.al. (1960) showed the effect of some alkylating agents on the incorporation of glycine-1-C^{14} into tissue protein in vitro. Ellis et.al. (1961) observed the effect of dinitrophenol on the uptake and incorporation of glycine in proteins. Similar types of study are carried out to see the effect of Jawaharene and Mitomycin C on the incorporation of acetate-2-C^{14}, glucose-U-C^{14}, alanine-1-C^{14} and glycine-1-C^{14} into the proteins of normal and SLT cells. Results in Tables 10-13 show that both these compounds inhibit the incorporation into proteins of tumour cells to a greater degree than that observed in the normal tissue. From Table 9 it can be observed that there is 1.6 times higher incorporation of C^{14} into the lipids and 5 times higher incorporation of C^{14} into proteins of tumour tissue when compared with normal thigh muscle. Tumour tissue is a fast growing tissue and there would be a faster turn over rate of all the tissue components. The higher incorporation in the tumour

tissue might be due to this fact and any inhibition would be more pronounced in faster growing tissue than in slow growing tissue. The two antibiotics have direct effect on protein synthesis. However, Jawaharene showed no effect at the concentrations used for Mitomycin C, so higher concentrations are used.

It should be emphasized that the experiments reported in this thesis are essentially short-term experiments and that effect of Jawaharene on tumour metabolism from different biochemical sides would further substantiate its antitumour property.

Clinical trials of Jawaharene on transplanted MFS, SLT and Ehrlich Ascites cells of mouse show promising results.

Besides, the inhibition studies with animal tumours can not establish anticancer values on human cases; animal tumours are not exact replica of human cancer; but the fact is transplanted animal cancer closely resembles human cancer pathologically, histologically and biochemically within certain limits. The significance of this work and results obtained justifies further biochemical studies and clinical trials on human patients.