CHAPTER IV

A QUANTITATIVE STUDY OF ACTIVITIES OF SUCCINATE DEHYDROGENASE AND ADENOSINE TRIPHOSPHATASE OF THE SUBMAXILLARY AND PAROTID SALIVARY GLANDS OF NORMAL AND ALLOXAN TREATED DIABETIC MALE ALBINO RATS.

Processes of synthesis of adenosine triphosphate (ATP) and utilisation of ATP run simultaneously in any live cells. Krebs cycle in the oxidative phosphorylation operates continuously to release paired hydrogen ions i.e. $2H^+$, which in tern pass through serially arranged factors of electron transport system (ETS) and produce ATP. At the same time hydrolysis of ATP is also obligatory part in the life of any active cell or tissue or gland. Succinate dehydrogenase (SDH) is the key enzyme of Krebs cycle, whereas ATPase is a reliable index of energy consuming activities of the cells or glands. Rate of operation of metabolic activities depends on availability of nutrient (glucose) from blood. In most of the glands pyruvate is made available by metabolising glucose which is obtained from ~~the uptake of~~ either glycogen or blood glucose. In fact Stadie (1954), has reported involvement of insulin in the metabolic reactions at the level of Krebs cycle. Decreased activity of SDH has been reported by Banerjee *et al.*, (1959) in mitochondria of certain organs of Scorbutic guinea pigs where the condition and activity of this mitochondrial enzyme improved after prolonged treatment of insulin. Decreased respiratory rate with ß-hydroxybutyrate and succinate as substrate is also shown by Boveries *et al.*, (1969) in the mitochondria of hepatic cells of alloxan treated diabetic rat. Alterations in the activities of certain enzymes like succinate dehydrogenase (SDH) ß-hydroxybutyrate dehydrogenase (BDH) and ∝-glycerphosphate dehydrogenase (∝-GPDH) have been extensively studied earlier in wound healing and regenerating liver and skin of normal and alloxan treated diabetic rats to understand influence of insulin on functional status of mitochondria in these structures (Kishnani, 1976; Kaṭuria, 1976) in our laboratory.

Several reports in the past have directly or indirectly suggested the presence of active transport in salivary glands (Schwartz *et al.*, 1963). Submaxillary gland which

is made up of heterogeneous mixture of mucous and serous acini posses active adenosine triphosphatase (ATPase) system (Schwartz, 1961; Bounting *et al.*, 1961). The parotid gland, which consists of serous acini show activities of most active Na^+, K^+- ATPase. Several workers have confirmed in their *in vitro* experiments that insulin exert a direct effect on carbohydrate metabolism. Vester and Stadie (1957) and Hall *et al.*, (1960), while studying the effect of insulin on oxidative phosphorylation in normal and diabetic rats have reported that activities of SDH in liver mitochondria is significantly lower than that of normal controls and that ATP formation is decreased to an even grater extent and insulin treatment restores the oxygen consumption and synthesis of ATP to normal level. Insulin could counteract the action of epinephrine and glucagon on plasma bound Na^+, K^+ - ATPase mediated by cAMP dependent negative modulation of a plasma membrane located protein kinase (Luly *et al.*, 1972; Barnabei *et al.*, 1973; Tria *et al.*, 1974). Na^+, K^+ dependent ATPase is widely accepted to be intimately associated with mechanism of transport of ions and as metabolites such as glucose, aminoacids etc. across glandular epithelial tissues (Tanaka, 1987). It is also known that Na^+, K^+ - ATPase activity contributes to the secretion of hypotonic saliva in the parotid glands of rats by facilitating the transport of sodium ions both intracellularly and para cellularly (Tadashi *et al.*, 1987). Na^+, K^+, ATPase has been shown to facilitate the transport of materials across the plasma membrane against the concentration gradient (Judah and Ahmed, 1964; Skou, 1965; Fransworth, 1972). Therefore any change in enzyme activity would reflect the flux of various substances including glucose across the plasma membrane from the salivary glands. Insulin has been shown to have specific direct regulatory effect on ATPase activity in rat adipocyte, plasma membrane, kidney, basolateral membrane, liver plasma membrane and sarcolemma of cardiac muscles in heart (Cohen *et al.*, 1980; Purrello *et al.*, 1982; Kasua *et al.*, 1982).

Since deficiency of insulin impairs oxidative metabolism, TCA cycle owing to the importance of these enzyme in regulating synthesis and secretory action and then composition of saliva, it was thought worth while to carry out comparative study of insulin deficiency on two different kinds of glands i.e. submaxillary and parotid salivary glands of normal and diabetic rats.

Materials and Methods :

Healthy male albino rats weighing 120 to 150 gms were selected in the present investigation. The rats were maintained in the laboratory conditions and were given balanced diet and water *ad libitum*. The treatments with saline and alloxan were given according to the method described in chapter I. The rats were sacrificed at selected intervals as described in the chapter I. The submaxillary and parotid glands were quickly excised, freed of connective tissue and were weighed on a Mettler balance. Using chilled mortar and pestle homogenate was prepared in chilled distilled water. The succinate dehydrogenase (SDH) activity was measured quantitatively using the chilled aqueous homogenate of both these glands separately employing the method of Kun and Abood (1949) using INT (indole nitrophenyl - triphenyl tetrazolium salt) as electron acceptor and the activity of SDH was expressed as µg formazoan formed/mg protein/30 minutes. Activity of total adenosine triphosphatase (ATPase) was measured employing the method described by Umbreit *et al.*, (1957) using Ouabain as inhibitor and expressed as µg phosphate released/ mg protein/10 minutes.

Protein content of homogenate was estimated employing the method described by Lowry *et al.* (1951).

Results :

Table IV and figure IVa and IVb present the data of activities of succinate dehydrogenase (SDH) and total adenosine triphosphatase (ATPase) in the submaxillary and parotid salivary glands of normal control and alloxan treated diabetic rats.

In normal and control rats submaxillary salivary glands showed higher activities of SDH than that of parotid gland. It is 22.53 and 12.49 µg formazoan formed/mg protein/30 minutes in submaxillary and parotid glands respectively. Both the salivary glands i.e. submaxillary and parotid showed considerably decreased value of succinate dehydrogenase activity at the level of $p < 0.001$ in diabetic state.

Table IV

Level of blood glucose, activities of succinate dehydrogenase (SDH) and total adenosine triphosphatase (ATPase) in submaxillary and parotid salivary glands of normal, control and diabetic male albino rats.
Mean ± S.D.

Physiological condition / animal	Blood glucose level [1]	SDH [2]		ATPase [3]	
		Submaxillary gland	parotid gland	Submaxillary gland	parotid gland
Normal	127.63 ± 8.96	22.53 ± 0.99	12.49 ± 0.64	62.43 ± 4.14	44.19 ± 1.82
Control	123.25 ± 10.95	21.49 ± 0.66	12.17 ± 1.27	61.30 ± 1.29	42.31 ± 1.39
Diabetic	257.04 ± 14.95	10.26 ± 1.36	6.27 ± 0.60	51.18 ± 1.89	36.75 ± 1.30
Significant (* p) at the level	p<0.001	p<0.001	p<0.001	p<0.005	p<0.001

1. mg blood glucose/100 ml blood (As an index of diabetic condition).
2. µg formazoan formed/mg protein/30min.
3. µg phosphate released/mg protein/10 min.

*p values refer to differences between normal and diabetic conditions.
The student's 't' test was used to analyse difference in means.

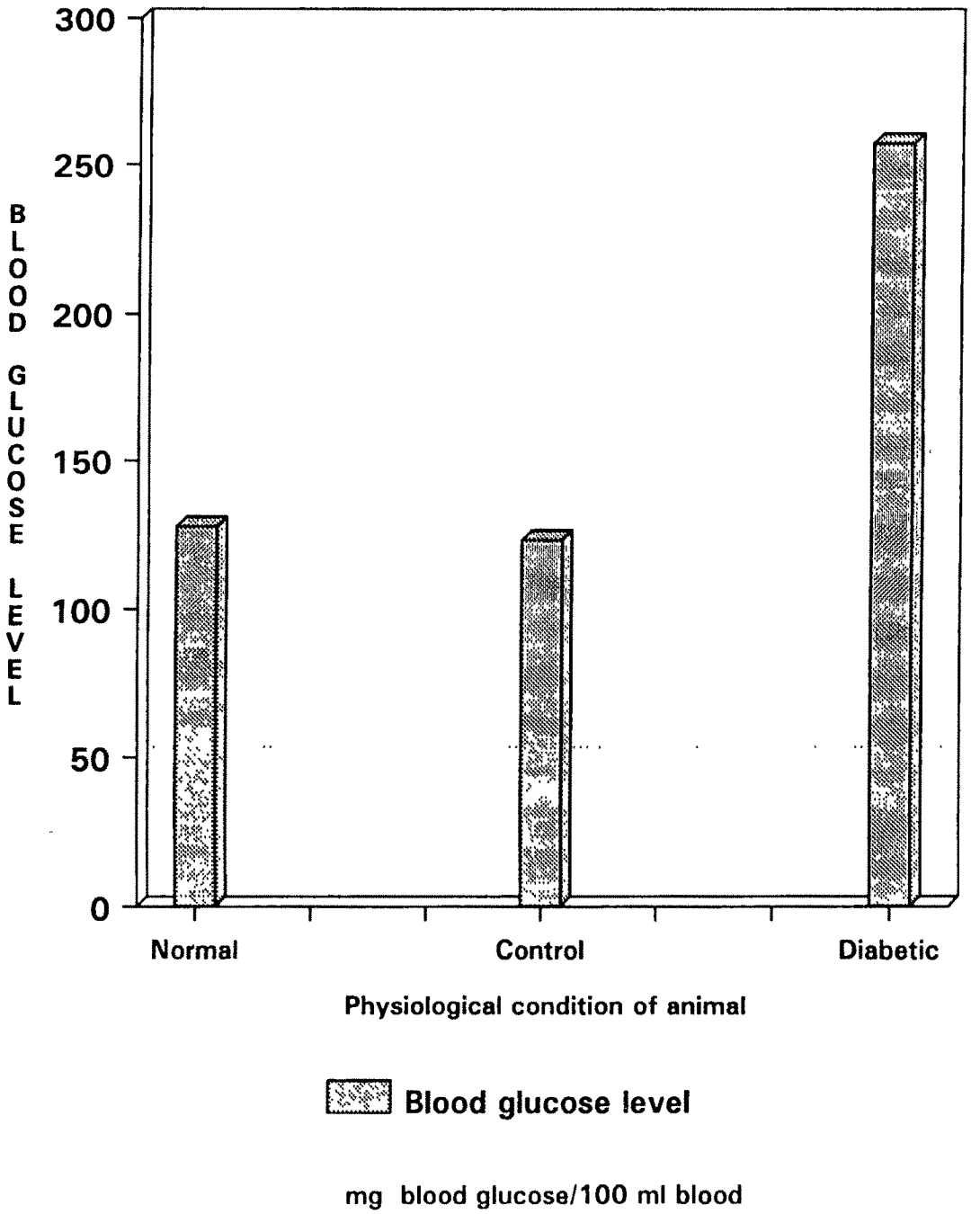

Fig.IVa. Graphic presentation of Blood Glucose Level of normal, control and diabetic male albino rats.

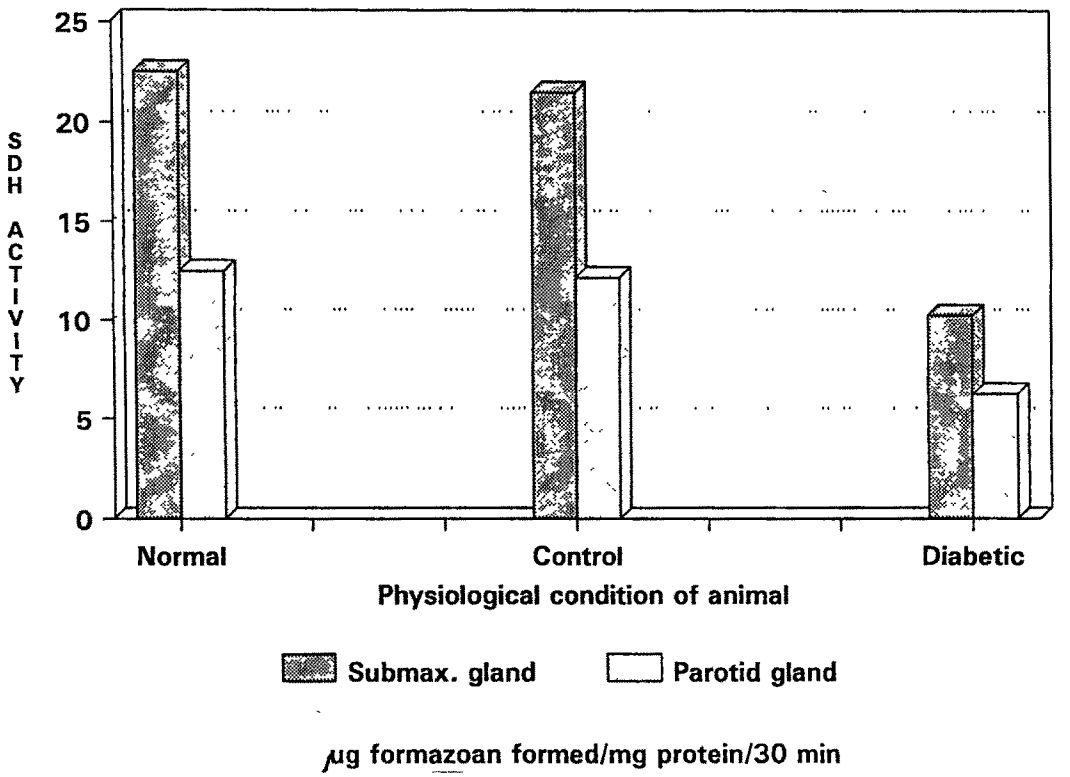

Fig.IVb. Graphic presentation of SDH Activity of submaxillary and parotid salivary glands of normal, control and diabetic male albino rats.

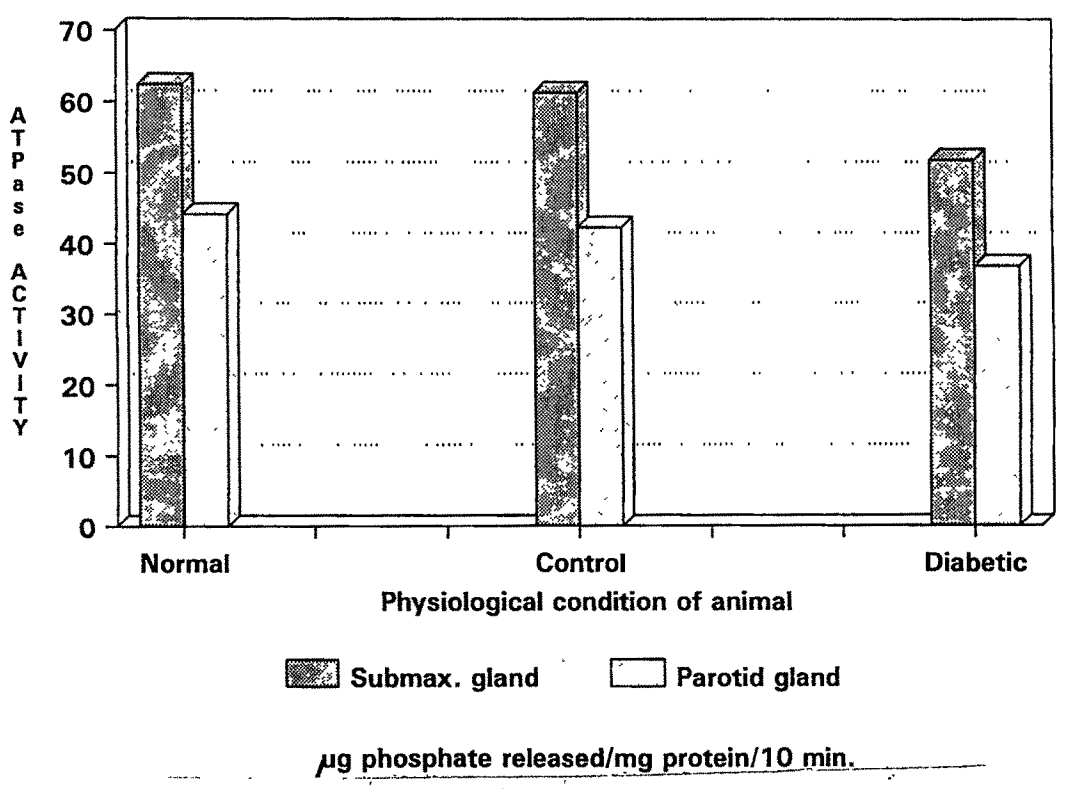

Fig.IVb. Graphic presentation of ATPase activity of submaxillary and parotid salivary glands of normal, control and diabetic male albino rats.

Activity of total ATPase of submaxillary gland is also higher than that of parotid gland in normal rats. Submaxillary gland showed 62.43 μg phosphate released/mg protein/10 min, whereas parotid showed 44.19 μg phosphate released/mg protein/10 min. Both the types of glands showed lower values of ATPase in diabetic condition. This decrease is statistically significant at the level of $p < 0.005$ in submaxillary and $p < 0.001$ in the parotid. Reduction is more in SDH activity, (around 50%), whereas it is less in ATPase activity (around 16-18%) in both the salivary glands of diabetic rats.

Discussion :

Dynamic state of equilibrium is an important feature of any vital tissues or organs in normal health and normal internal as well as external circumstances. Many factors are involved in maintaining this state of equilibrium and several factors deviate this ideal condition of many normal functioning and healthy organs. In general, rates of energy generating system, consumption of energy and amount of available energy in the vital cells decide the functioning of tissues or organ. Salivary glands are considered as very active and quick secretory glands. Two types of acini and their existence either as only clusters of serous or only clusters of mucous or as clusters of two kinds of acini have made the study of these glands interesting since several years. The biochemical nature of secretion of two types of acini differ. Metabolic machinery of both mucous and serous types of cells is also different and several neuronal as well as hormonal factors have specific and different effects on serous and mucous type of acini and even tubular parts of these salivary glands.

Many investigators in their *in vitro* experiment have confirmed that insulin exerts direct effect on oxidation of carbohydrate. Vester and Stadie (1957) and Hall *et al.*, (1960) have reported that oxygen consumption of mitochondria of liver of diabetic rats is significantly lower than that of normal rats. This study suggest that insulin is essential for maintaining normal oxidative phosphorylation. Deficiency of insulin also leads to reduced ATP synthesis. Stadie (1954), has also reported stimulatory effect or positive role of insulin in metabolic reactions of Krebs cycle. Banerjee *et al.*, (1959) has observed improvement of SDH activity in scorbutic guinea pigs after a prolonged treatment of insulin, such stimulatory effect of insulin directly on

mitochondrial function of hepatic cells of rat has been suggested by Schafter and Nagel (1968). These investigators observed increased activity of SDH and BDH in mitochondria of hepatocytes of rats. Bovericee *et al.*, (1969) studied mitochondrial function in insulin deficiency, where the investigators observed decreased activities of succinate dehydrogenase and ß-hydroxybutyrate in liver cells of diabetic rats. Insulin regulates the pyruvate dehydrogenase complex that produces acetyl CO-A necessary for lipid synthesis (Krahl, 1974; Denton *et al.*, 1971; Huston *et al.*, 1978; Baxter and Coore, 1978; Mukherjee and Jungas, 1975; Paetzke Brunner *et al.*, 1979; Sakamoto and Kuzuya 1979). Insulin activates this enzyme complex by dephosphorylation (Krahl, 1974; Denton *et al.*, 1971; Huston *et al.*, 1978; Baxter and Coore, 1978). Not only generation of ATP is affected but several reports indicate that synthesis of the secretory material is also reduced. Palla *et al.*, (1967) have observed decreased biosynthetic activity of amylase enzyme molecules in the pancreas and parotid salivary glands of diabetic rats. Pillai *et al.* (1989), have also observed reduced activity of amylase and trypsin in submandibular salivary glands of alloxan treated diabetic rats. Similarly, deficiency of insulin in all probability must be affecting even synthesis and secretion of mucin containing several peptides, glycoproteins and even epidermal growth factor (EGF). In fact saliva, through some of its common constituents of mucin do have certain inhibitory action on growth of bacteria or putrification of food particles in the mouth. Even many carnivorous and other mammals do lick wounds for cleaning it. This natural habit prevent chances for infection of wound. Probably this serve two purposes, some of the peptides or glycoproteins prevent the infection of wound and factors like EGF speed up the wound healing process. Synthesis of all these macromolecules such as amylase in serous acini and various components of mucin in mucous acini requires energy which is available from the common energy rich molecule ATP. Naturally, ATPase induced hydrolysis of ATP is also considered as an important feature of normally functioning acini (serous as well as mucous) of salivary glands in normal condition of animals. Consequently, the results presented earlier (Chapter I) indicated that activity of amylase has been reduced in both the types of salivary glands of diabetic rats. Even higher values of reserved metabolites such as glycogen and lipids in both the types of salivary glands of diabetic rats (chapter II) indicated the reduced utilisation of these metabolites due to deficiency of insulin. Now the results of quantitative assay of succinate dehydrogenase and adenosine triphosphatase showed decreased value in both types of salivary glands of diabetic rats. These results indicate subnormal function of mitochondria due to absence of insulin. In this condition one can expect lower value of key enzyme of Krebs cycle i.e. SDH activity.

Simultaneously, decreased demand of energy required for synthesis of many macromolecules in both the types of gland cells (serous and mucous) of salivary glands is expected in such condition of deficiency of insulin and coincidentally decreased or reduced value of activity of ATPase in both the type of salivary glands indicate a subnormal activity of salivary glands of diabetic rats.

It could be concluded that the decreased activities of succinate dehydrogenase (SDH) and total Adenosine triphosphatase (ATPase) in both the types of salivary glands of diabetic rats could be due to subnormal energy generating activity of Krebs cycle under absolute deficiency of insulin. Similarly slow synthesis of macromolecules such as mucopolysaccharides, glycoproteins, epithelial growth factor (E G F), α-amylase, sialic acid etc and possible qualitative and quantitative changes in secretory function of these glands of diabetic animals reduced the demand of energy due to absence of stimulatory action of insulin could be also visualized from significant decline in total ATPase activity in these glands of diabetic animals.