

## CHAPTER II

### **A QUANTITATIVE STUDY OF WATER CONTENTS, GLYCOGEN, PROTEINS AND LIPIDS OF SUBMAXILLARY AND PAROTID SALIVARY GLANDS OF NORMAL AND ALLOXAN TREATED DIABETIC MALE ALBINO RATS.**

Water is the most abundant inorganic material in the body of organisms. It makes up almost seventy percent or more of the weight of most of the fresh tissues except bones and cartilages which contain comparatively less amount of water. It is the main constituent of intracellular as well as extracellular compartments. It is the common medium for uptake of metabolites as well as secretion of glandular cells. All the biochemical metabolic activities take place in the aquatic medium which is provided by water content of the cell. Salivary secretion involves the epithelial transport of water and electrolytes and the exocytosis of protein both of which are under neural control (Garrette, 1987). Salivary glands are such that their functions principally dependent on water and salt concentration. Water is extremely important for these salivary glands and it plays many important roles for maintaining structure as well as functions of salivary glands. Any alterations in structure and/or function of these salivary glands may reflect on its water content. In salivary glands the situation is further complicated because not only is there simultaneous protein and mucopolysaccharide synthesis and transport of water and ions but there is usually present also a special morphological entity, the striated duct, which is associated with transportation of water and ions (Junqueira *et al.*, 1964). One possible explanation implicates dehydration and electrolyte depletion which occurs in the decompensated diabetic state. Under such circumstances chemical gradient favourable to the absorption of water and sodium might be created in the diabetic animals. Since losses of fluid and electrolytes progresses with the passage of time, water and electrolyte absorption should be progressively augmented in these animals. Menghi *et al.*, (1990), reported the water release from the sublingual, parotid and submandibular glands of male and female rats by thermal analysis in order to detect the total water content in different types of glands. In addition these authors have indicated the evidence of a sexual dimorphism in the rat sublingual gland. Thus water content of these two glands depend on many factors, such as uptake of water from blood with or without metabolites and utilization of water in cells as well as volume and

concentration of their secretions (saliva). In the present investigation two different types of salivary glands have been selected. Parotid gland is a pure serous type which secretes watery secretion containing  $\alpha$ -amylase and the submaxillary gland is mixed type which secretes an iso mixture of  $\alpha$ -amylase and mucopolysaccharides. Present chapter deals with the study of water content of these glands comparatively in normal conditions as well as in diabetic condition.

Various factors are known to play important roles in regulation of over all carbohydrate metabolism (Northrup and Parks, 1964; Northrup, 1968; Hornbrook, 1970; Froberg *et al.*, 1975). Carbohydrate metabolism in various tissues is known to be influenced by the several circulating hormones (Matute and kalkhoff, 1973; Froberg *et al.*, 1975; Whitton and Hems, 1976; Martirosyan 1980; Meshchishen *et al.*, 1980; Balasubramanian *et al.*, 1981; Hiroyuk *et al.*, 1981; Malbon and Camphell, 1982). The common and main fuel for most of the living cells of any tissue or gland is glucose, which is readily available from blood. Hepatic cells which are the main cells in interconversion of glucose and glycogen, play very significant role in glucoregulation. Muscle fibres on the other hand synthesize glycogen as a stored material to generate energy required for muscular action while other organs such as kidney (Mehta, 1985; Pilo and Mehta, 1988) and skin (Patel and Pilo, 1979) also have metabolic machinery and capacity to synthesize and store at least little amount of glycogen. There are reports that even uptake of glucose and synthesis as well as hydrolysis and thus content of glycogen etc show alterations under influence of several neuronal as well as hormonal factors in salivary glands (Palla *et al.*, 1967; Anderson *et al.*, 1979). Due to the importance of glycogen as the primary energy source for most of the animal tissues both glycogen as well as glycolytic enzymes have been extensively studied in the various vertebrate tissues in different physiological conditions (Mencini, 1948; Montagna, 1948; Bergman, 1960; Grillo, 1961; Falin, 1961; Cosmos, 1966). Hypophysectomy leads to a fall in the blood sugar and hepatic glycogen levels, while administration of prolactin and corticosterone to hypophysectomized lizards was effective in restoring both the parameters to normal levels (Callard and Chan, 1972). Glycogen synthase, exists in a less phosphorylated I-form and more phosphorylated D form. The less phosphorylated form synthase I is considered to be the physiologically active form *in vivo* (Villar-Palasio *et al.*, 1968, 1970; Soderling *et al.*, 1974).

Glycogen phosphorylase, the rate limiting enzyme in glycogen degradation also exists in a phosphorylated form, phosphorylase-a and a dephosphorylated form phosphorylase-b. Phosphorylase-a is considered to be the physiologically active form. However, phosphorylase-b can be stimulated by the allosteric activator cAMP and thus be catalytically active *in vivo* as well (Nuttall, 1972). Activation of synthase under influence of insulin was demonstrated in an openchested rat heart preparation by William and Mayer (1966). Glycogen synthase an enzyme that is concerned with the synthesis of glycogen is reported to be present in the muscle and liver of vertebrates (Leloir and Cardini, 1957).

It is reported that in insulin-deficient diabetic animals carbohydrate metabolism of the liver is generally affected (Renold *et al.*, 1953, 1955; Ashmore *et al.*, 1957). It has been pointed out that hepatic glycogen content get depleted in uncontrolled diabetes (Bondy *et al.*, 1949; Zimmerman *et al.*, 1950; Goodman, 1953). In the liver, one metabolic sequence of insulin deficient diabetes mellitus is an impairment in the ability to synthesize and store glycogen (Hornbrook, 1970; Whitton and Hems, 1975). Several studies have reported about histochemical localization as well as quantitative assay of activity of glycogen synthase in the liver of diabetic animals (Gold, 1970; Langdon and Curnow, 1983). Both glycogen synthase and glycogen phosphorylase exist in active as well as inactive forms. The interconversion between these forms are controlled by protein kinase catalyzed phosphorylation and phosphoprotein phosphatase catalyzed dephosphorylation (Curnow *et al.*, 1979). Activation of synthase is catalyzed by synthase phosphatase enzyme and overall regulation is effected by hormones particularly insulin and metabolites in particular, blood glucose level as well as glycogen content in the tissue or gland cells (Curnow *et al.*, 1979).

Prasannan (1973), has reported that liver glycogen diminished in alloxan diabetic rats. Reports are there which indicate the decreased activities of G-6-PDH (Glucose-6-phosphate dehydrogenase) and LDH (lactate dehydrogenase) in the liver during alloxan induced diabetes (Ralph *et al.*, 1964; Chung and Hong, 1968; Germanyuk and Gulava, 1970). Gold (1970), reported that alloxan diabetes results in a loss of activity of the glycogen synthase in the liver. Insulin treatment of diabetic rats restores glycogen synthase activity to almost normal level. The glycogen phosphorylase activity and metabolism of glycogen have already been correlated ever since 1943

(Shapiro and Wertheimer, 1943) and further corroborated later in 1960 by Stetten and Stetten.

Taking in to account such varied reports, it is apparent that there occur several alterations in carbohydrate metabolism of wide variety of organs and tissues of many animals. However, such reports are not easily available for salivary glands. Smith and Frommer (1975), have shown that steroid hormone leads to an increase in carboxylated mucosubstances within the acini as well as glandular tubules of mouse submandibular glands.

Proteins are the building blocks of the living body and they play a salient role in the maintenance of structural and functional integrity of the different tissue or gland cells.

Leathem (1970), reported that certain hormones stimulated protein metabolism in some organs while inhibitory effects may be observable in others. Rat salivary glands are most frequently utilized model for study (*in vivo*) of exocrine protein and electrolyte secretion (Young and Schneyer, 1981). Salivary glands synthesize and secrete characteristic complements of proteins (Ball *et al.*, 1988a and 1988b). Two of the major salivary products synthesized by the acinar cells of the submandibular salivary gland (SMG) are salivary mucin (Tabak *et al.*, 1985) and a family of glutamine and glutamic acid rich proteins, GRP (Mirels *et al.*, 1987; Heinrich and Habener, 1987). Secretion from the rat parotid gland is controlled by the neurosecretions of parasympathetic and sympathetic nerves. The parasympathetic nerve is generally considered to provide the main impetus for fluid secretion whilst the protein content of this secretion may be altered by the super-imposition of impulses from the sympathetic supply (Emmelin, 1987). Stimulation of sympathetic nerves induce small volume of secretion of protein rich parotid saliva where as stimulation of parasympathetic nerve induces secretion of comparatively large volume of saliva of low protein concentration (Garrett and Thulin, 1975; Anderson *et al.*, 1984).

The non-parallel effects of diabetes on parotid and submandibular glands secretory proteins are even more clearly shown by the influences in electrophoretic patterns (Carbone and Sweeney, 1962). These investigators demonstrated 30-40 % decrease in total protein content of the submandibular saliva with no disproportionate changes in any one protein band of particular interest i.e there were no changes in a class of secretory proteins unique to salivary secretions. The proline rich proteins, constitute approximately 80 % of total protein in parotid saliva (Robinovitch *et al.* 1977). In the present investigation, estimation of protein content was carried out in two different types of salivary glands, with an aim to have a comparative idea of normal protein levels as well as nature of alterations between these two glands and to know whether they are parallel or non-parallel in diabetic condition.

Hyperlipidemia is a metabolic complication of both clinical (Albrink *et al.*, 1958) and experimental diabetes (Bierman *et al.*, 1975). In animals, the administration of diabetogenic doses of streptozotocin induces hypertriglyceridemia (Schein *et al.*, 1971; Bar-on *et al.*, 1975). Probably this is due to reduced up take and utilization of lipids by tissues. Derrangement, to varying degrees, in the metabolism of glucose, lipids and proteins etc. is the characteristic feature of diabetes mellitus (Abrams *et al.*, 1982; Chua *et al.*, 1983). Alterations of membrane phospholipids and fatty acid composition have been found in many endocrine disorders, such as diabetes mellitus, hyperthyroidism and hypothyroidism (Faas *et al.*, 1980) and other conditions such as aging (Schroeder *et al.*, 1984) and consumption of diets supplemented with fish or vegetable oils (Deschrijver *et al.*, 1982). In diabetes mellitus, these alterations have been described in a variety of tissues including liver, heart, kidney, blood and aorta (Holman *et al.*, 1983). An increase in the compound lipids in liver of alloxan treated diabetic rat has been observed. Duncan (1959), found fatty deposits in the liver of untreated diabetic patients and these deposits disappeared quickly when the disease was controlled by insulin therapy. Overall general significance of lipid metabolites and their metabolism on the functional status of various tissues has been reported (George and Jyoti, 1957; Fredrikson and Gordon, 1958; Ambadkar 1969; Ambadkar and Gangaramani, 1976; Valettle *et al.*, 1978; Patsch *et al.*, 1980; Schuler *et al.*, 1981).

In both insulin dependent and non insulin dependent diabetes, plasma triacylglycerols and very low density lipoprotein contents are increased, where as

high-density lipoproteins are often decreased (Schonfeld, 1985; Laakso *et al.*, 1985). Van TOIA (1977), injected streptozotocin treated rats with labelled very low-density lipoproteins (VLDL), and found that the fractional clearance rate of radio active triacylglycerols was severely reduced. High fat diets are known to alter plasma lipids and other metabolites compared with insulin - deficient rats on control diets (Baxter *et al*, 1980). In addition, fat is known to influence the m-RNA translation and synthesis of apolipoprotein, A IV by the intestine (Apfelbaum *et al.*, 1987), Malathy *et al* (1972), studied the changes in glycosaminoglycans and lipids in the aorta, kidney, skin and retina of alloxan diabetic rats, the increase in phospholipid was moderate in the skin, but slight increase in the kidney, aorta and retina.

In the present work, quantitative estimation of total lipid content has been carried out in submaxillary and parotid salivary glands with an aim to have comparative evaluation of lipids content in normal conditions as well as nature of alterations in diabetic condition in these two different types of salivary glands of male albino rats.

## **Materials and Methods**

In the present study healthy male albino rats of 120 gms to 150 gms were kept under laboratory conditions. They were maintained on a balanced animal diet and water *ad libitum*.

The animals were divided into three groups, the saline injections and the alloxan treatment was given to the different groups of rats as described in the Chapter I. The respective groups of rats were sacrificed on the selected intervals for each batch as described in the Chapter I. The rats were killed under mild ether anaesthesia by cervical dislocation. Both the sides of submaxillary and parotid glands were quickly removed, freed of connective tissue and weighed on the Mettler balance. One side of both the parotid and submaxillary glands were kept for drying in the oven at 60°C temperature for two-three days after keeping glands in preweighed test tubes. Once

the tissue was completely dried the weight of the glands was taken and the tissue was kept in the oven for further drying till the constant weight of the tubes containing tissue was obtained. Thus the total water content of the submaxillary and parotid glands was estimated by simple gravimetric method and the total water content was calculated and expressed in terms of mg%, i.e. mg water/100 mg of fresh gland as shown in the table II. The total glycogen content was estimated quantitatively employing the method of Seifter *et al.* (1950) using anthrone reagent. The total glycogen content estimated was expressed as mg% i.e. mg glycogen/100 mg of fresh gland as shown in the table II. The total lipid content was estimated using extraction method from the pre weighed dry tissue employing the method suggested by Folch *et al.*, (1957) using mixture of chloroform, methanol (2:1) as lipid extracting medium. The filtrate of each sample was filtered carefully through filter papers separately. Complete extraction was carried out by repeating 4 to 5 times filtrate collected in preweighed labelled test tube. The weight of the residue and the filter papers were taken carefully. Weight of the total lipid content was taken after complete evaporation of chloroform-methanol from the filtrate. Total lipid content of glands is expressed as mg lipid/100 mg of fresh gland, as shown in the table II. The total protein content was estimated by following the method of Lowry *et al.*, (1951), and it is expressed as mg protein/100 mg of fresh gland, as shown in the table II. Statistical analysis was done using the Student's 't' test.

### **Results :**

The data obtained are presented in the table II and Fig. IIa, IIb and IIc. The level of blood glucose in normal, control and diabetic rats are 128.06, 122.95 and 257.02 mg/100 ml of blood respectively. The data included here which is a supporting data as an index of diabetic condition. The total water content of submaxillary salivary glands in the normal, control and diabetic rats are 73.08, 73.49 and 72.16 mg water/100 mg weight of fresh gland respectively and the total water content of the parotid gland in the same groups of animals are 68.21, 68.84 and 70.48 mg water/100 mg weight of fresh gland respectively. In normal condition parotid gland showed lower value than that of submaxillary. Water content of both the types of glands did not show any significant alterations in diabetic conditions. The total glycogen content of submaxillary gland, the normal, control and diabetic rats are 0.041, 0.040 and 0.055 mg glycogen/100 mg of fresh glands respectively, and that of parotid gland in the same

Table II

Level of blood glucose, total water, glycogen, protein and lipid content of submaxillary and parotid glands of normal, control and diabetic male albino rats.  
Mean  $\pm$  SD

Physiological condition of animal	1 Blood glucose level		2 Water content		3 Glycogen content		4 Protein content		5 lipid content		Significant at the level
	submax. gland	parotid gland	submax. gland	parotid gland	submax. gland	parotid gland	submax. gland	parotid gland	submax. gland	parotid gland	
Normal	128.06 $\pm$ 9.25	73.08 $\pm$ 1.44	68.21 $\pm$ 1.09	0.041 $\pm$ 0.005	0.043 $\pm$ 0.004	17.24 $\pm$ 0.73	16.08 $\pm$ 0.49	3.84 $\pm$ 0.47	5.68 $\pm$ 0.84		
Control	122.95 $\pm$ 11.23	73.49 $\pm$ 0.46	68.84 $\pm$ 0.46	0.040 $\pm$ 0.001	0.042 $\pm$ 0.002	16.99 $\pm$ 0.54	15.94 $\pm$ 0.14	3.95 $\pm$ 0.25	5.55 $\pm$ 0.68		
Diabetic	257.02 $\pm$ 15.36	72.16 $\pm$ 1.06	70.48 $\pm$ 2.38	0.055 $\pm$ 0.002	0.056 $\pm$ 0.003	16.51 $\pm$ 0.27	15.79 $\pm$ 0.41	7.80 $\pm$ 0.76	8.80 $\pm$ 0.97		P<0.001

1. mg glucose/100 ml blood (as an index of diabetic condition)

2. mg water/100 mg fresh gland

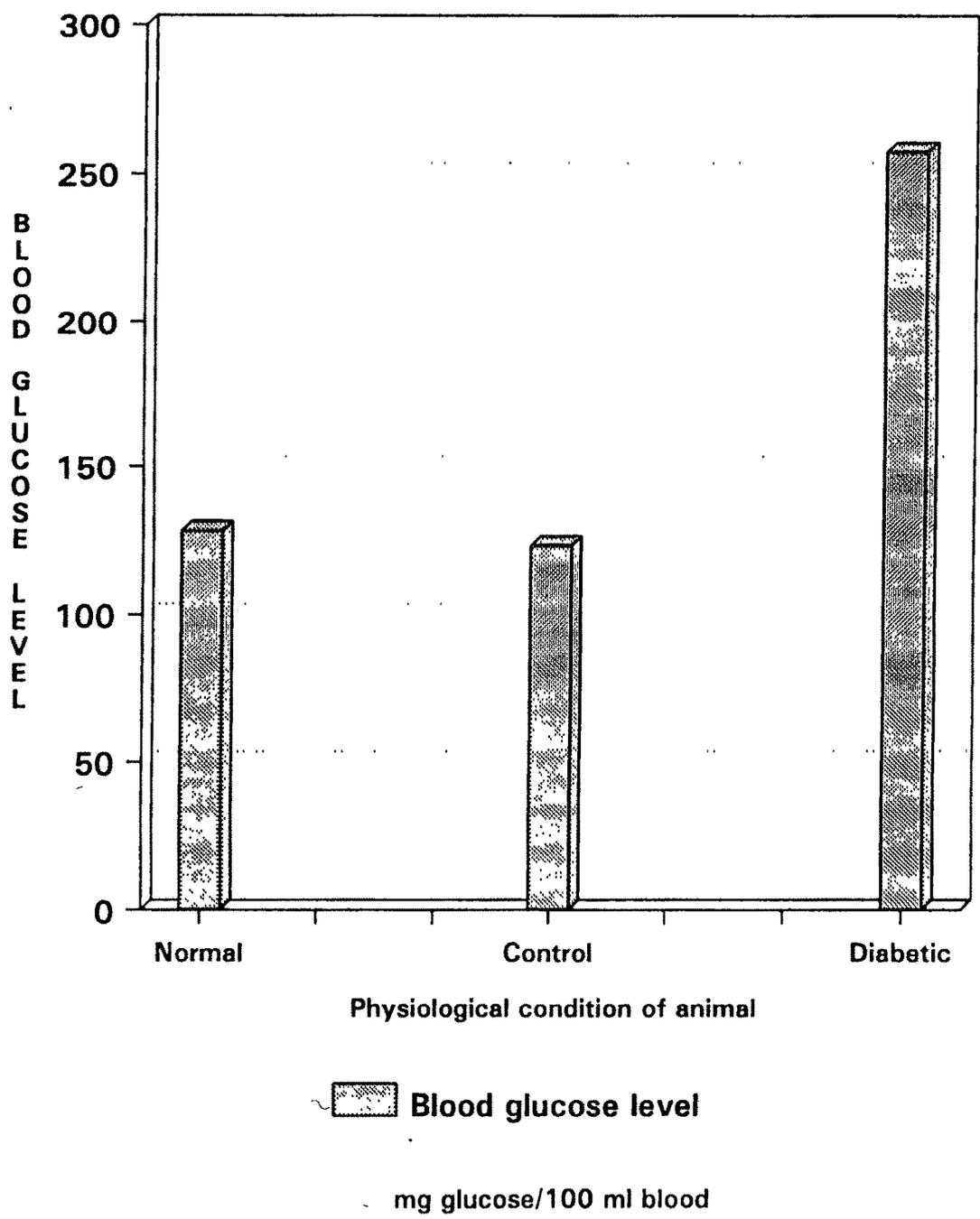
3. mg glycogen/100 mg fresh gland

4. mg protein/100 mg fresh gland

5. mg lipid/100 mg fresh gland

\*P values refer to differences between normal and diabetic conditions.

The student's 't' test was used to analyse differences in means.



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

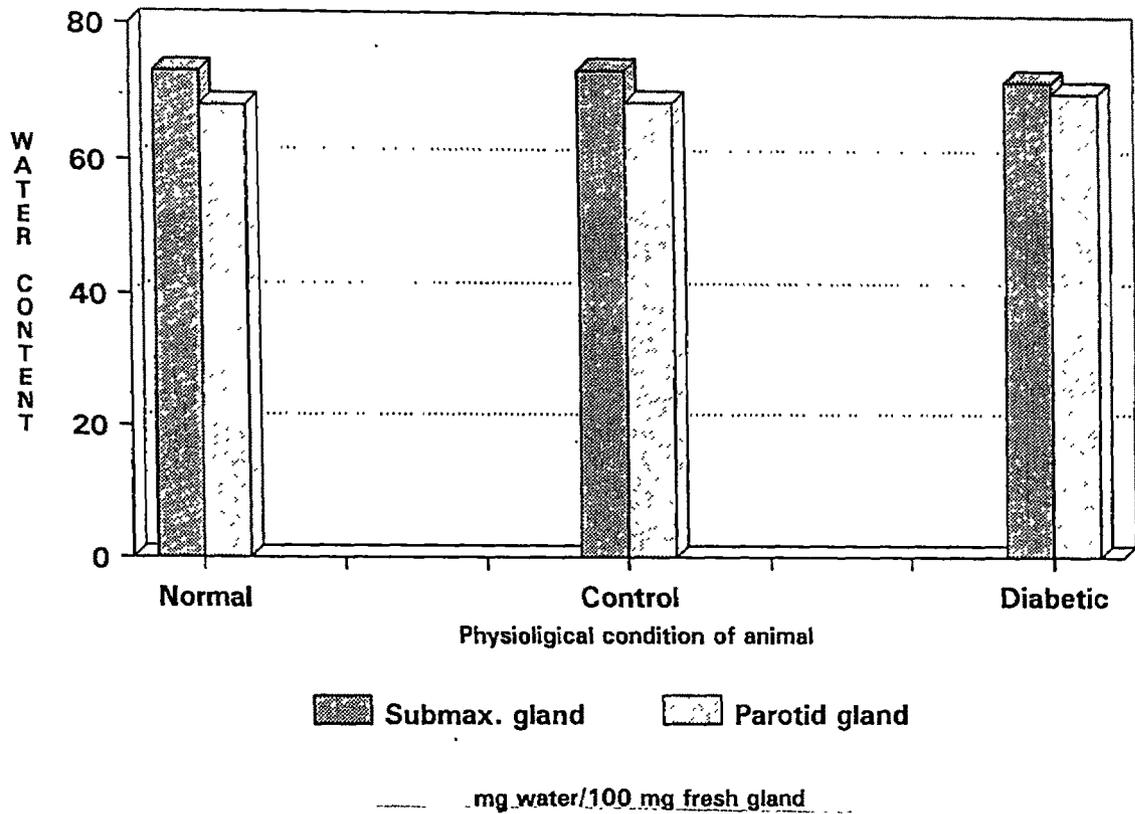


Fig.IIb. Graphic presentation of water content of submaxillary and parotid salivary glands of normal, control and diabetic male albino rats.

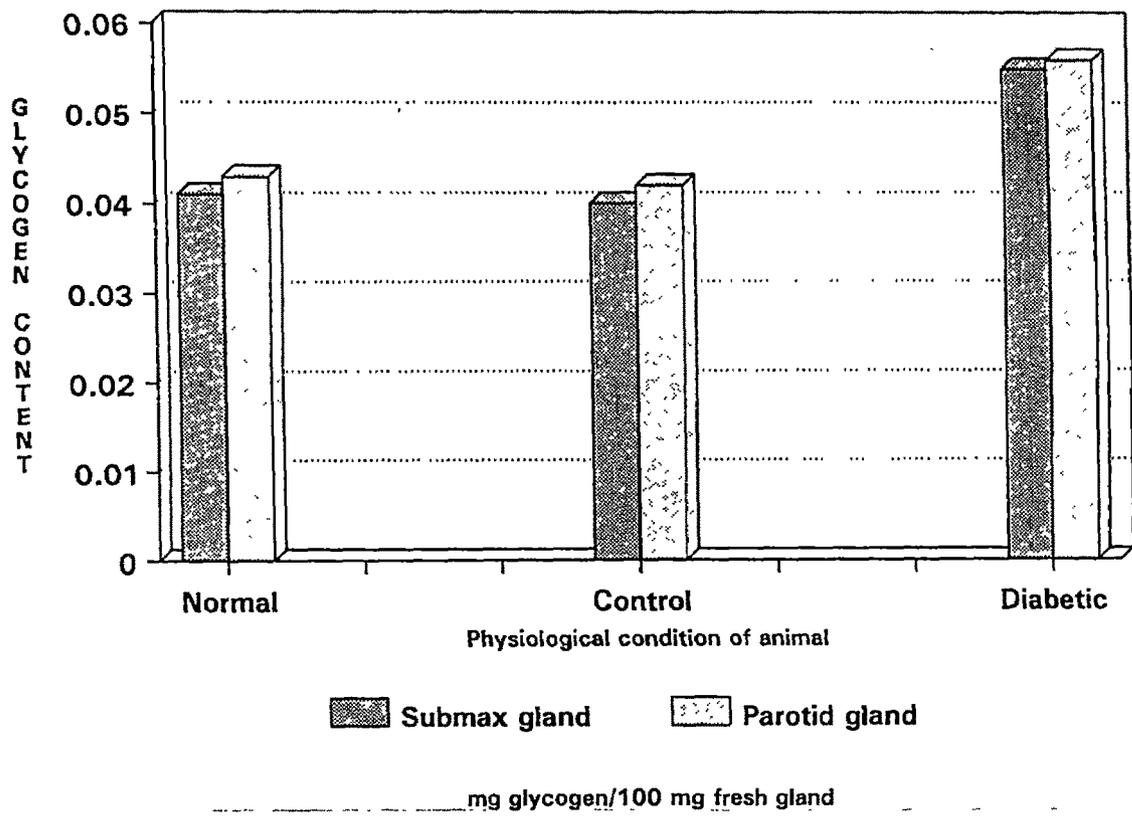


Fig.IIb. Graphic presentation of glycogen content of submaxillary and parotid salivary glands of normal, control and diabetic male albino rats.

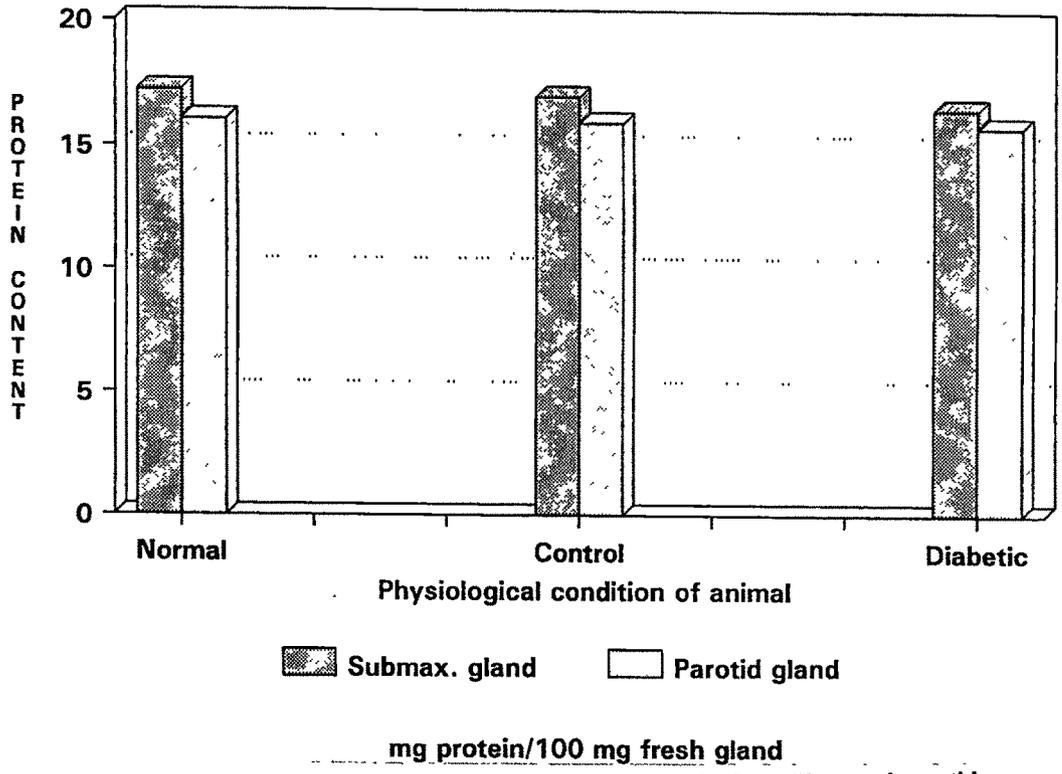


Fig.IIc. Graphic presentation of protein content of submaxillary and parotid salivary glands of normal, control and diabetic male albino rats.

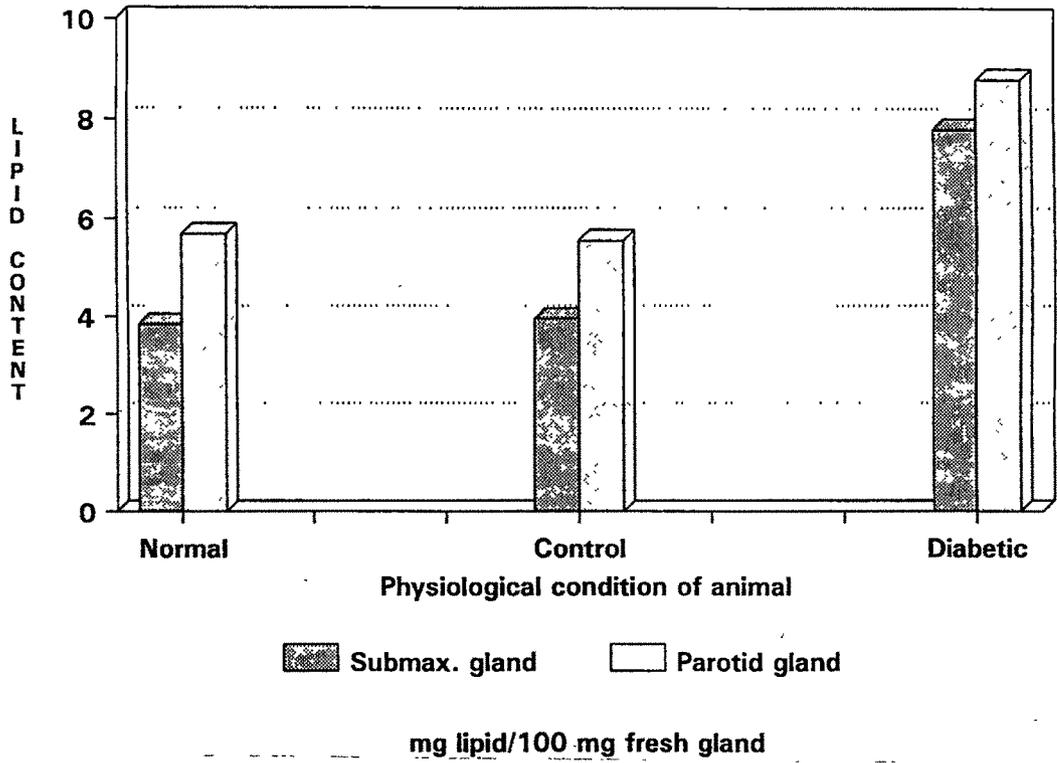


Fig.IIc. Graphic presentation of lipid content of submaxillary and parotid salivary glands of normal, control and diabetic male albino rats.

groups are 0.043, 0.042 and 0.056 mg glycogen/100 mg of fresh glands respectively. Both the types of glands showed higher glycogen content in the diabetic condition than the normal. An increase in submaxillary gland is significant at the level  $p < 0.001$ , where as that in parotid it is at the level  $p < 0.03$ .

The total protein content of the submaxillary gland in normal, control and diabetic groups of rats are, 17.24, 16.99 and 16.51 mg protein/100 mg of the fresh gland and that of parotid gland in the same groups of rats are 16.08, 15.94 and 15.79 mg protein/100 mg of fresh gland respectively. The submaxillary gland showed non-significant decrease while the parotid gland showed slight decrease in its protein content in the diabetic condition, which is significant at the level ( $P < 0.002$ ).

The total lipid content of the submaxillary gland in normal, control and diabetic groups of rats are 3.84, 3.95 and 7.80 mg lipid/100 mg of fresh gland and that of parotid gland in the similar groups of rats are 5.68, 5.55 and 8.80 mg lipid/100 mg of fresh gland as shown in the table II. Thus both the salivary glands showed significant higher lipid content in the diabetic condition. In normal condition parotid gland showed comparatively more lipid than submaxillary.

### **Discussion :**

The water content of any secretory cells is determined by the uptake of water from blood with or without metabolites, as well as release of water along with its secretion. Salivary glands are known for their faster secretory actions. Again in mammals, there are different types of salivary glands with definite types of acini (serous and mucous) and even with definite different and specific nature or composition of saliva. In many carnivorous animals, active salivary function is observed at the time of ingestion as well as during panting in summer for thermoregulation to a certain extent. Being voracious feeder secretion of salivary gland in rodents like rats and mice is also very high. Effects of neuronal factors on the secretion are known since many years (Bloom *et al.*, 1981). Active cholinergic nerves or parasympathetic nerves stimulate active secretion or output of water and electrolytes by parotid gland, whereas sympathetic stimulatory tone produce viscous saliva with less water (Bell,

Davidson and Smith, 1967). Since last one decade or so certain groups of investigators have focussed their attention to study influence of sex hormones on salivary glands (Schuermeyer *et al.*, 1984; Kasayama *et al.*, 1989; Desai, 1989). Salivary glands have been investigated in relation to sexual differences in male and female animals (Lacassagne, 1940a, 1940b). Menghi *et al.*, (1989 and 1990), have studied differentiation of mice and rat salivary glands in relation to sex of animals as well as composition or nature of their secretion emphasizing water content in saliva. Seaton and Fahmy (1979) and Walker *et al.*, (1981) have also studied occurrence of steroid receptors and metabolic pathways of these hormones. Receptors of one or other kind of steroid hormones are distributed on various kinds of glands and even muscle fibres. Similarly insulin receptors are also widely distributed throughout the body and hence no single organ or tissue shows normal functional status in conditions such as insulin deficiency or diabetes mellitus. This endocrine disorder induces a complex series of metabolic dearrangments in almost all the parts of digestive system as well as other systems and glands, such as kidney, heart etc. Insulin action is very dominant on reabsorption of water electrolytes and metabolites in uriniferous tubules as kidneys are more concerned with osmoregulation and excretion. Kidney is the final outlet for water and electrolytes where as output of salivary glands once again goes to the gut. Levinson *et al.* (1970), have reported that jejunal and ilial segments showed increased sugar absorption but they failed to notice significant change in salt and water absorption in the same intestinal segments. From the results of present investigation it could be seen that total water content of parotid gland is slightly lower than that of submaxillary gland. Water content of submaxillary gland is 73.08% and that of parotid gland is 68.21 % in normal condition of animals. Both the salivary glands showed non-significant and non-parallel alterations in the diabetic condition. These results indicate that rate of uptake of water into gland cells and rate of release of water along with secretion are identical and run parallel. Hence glands failed to show significant alterations in water content. Coincidentally slightly lower water content of parotid in normal condition is compensated by higher lipid content in parotid gland. It is interesting to point out here that even water content of adipose tissue and liver depletes when fat deposition increases in premigratory phase of Rosy Pastors and Wagtail (Pilo, 1967).

Capacity of uptake of glucose and synthesis as well as storage of glycogen varies in different glands. Hepatic cells are not only very active in uptake as well as release

of glucose but also for active synthesis as well as hydrolysis of glycogen. This is because of its basic function in glucostasis. Muscle fibres also contain glycogen as a reserved metabolites for generating energy for muscular action. Similarly each and every cell in the body contain little amount of glycogen as readily available reserve metabolites. In alloxan treated rats, severe deficiency or absence of insulin will affect uptake of glucose and carbohydrate metabolism in serous as well as mucous types of cells of the salivary glands.

There are several reports regarding influence of circulating hormones on carbohydrate metabolism in various tissues. Key enzyme or rate limiting enzyme of gluco-genesis is glycogen synthetase and rate limiting enzyme of glycogenolysis is phosphorylase. Activities of these enzymes in their turn are influenced by hormones as well as neuronal factors (Sutherland and Rall, 1960; Krebs and Fisher, 1962; Lerner, 1966). Increase in glycogen content of submaxillary gland of albino rats after castration was observed by Desai in 1989.

In the present study both the types of salivary glands showed higher value of glycogen content in the diabetic condition. Normally hexokinase and glycogen synthetase enzyme are more sensitive to insulin in the mammalian liver (Pilo and Patel, 1978). Three isoenzymes of hexokinase exist in mammalian tissues (McGilvery, 1972). Different tissues contain one or two different isoenzymes of hexokinase. Liver contain all the three types. Type II is sensitive to insulin (Skeletal muscle and adipose tissue have more of this type), while type I is insensitive to insulin. Brain and kidney have I and heart contains both type I and II. Perhaps the salivary glands contain hexokinase I and thus uptake of glucose from blood to salivary gland cells is not affected. However, basic function of salivary glands, to synthesize and secrete different components of saliva, is affected or reduced in salivary glands of diabetic rats. Parotid glands of diabetic rats showed reduced amylase activity (Chapter I). Similarly, one can expect even reduced synthesis of various constituents of mucin in mucous cells of submaxillary glands also. Mucin is a complex mucopolysacchride. When its synthesis is reduced, naturally utilization of glucose is also reduced in submaxillary glands. Thus higher content of glycogen in the salivary glands of diabetic rats than that of normal rats is not due to its increased synthesis but probably due to its reduced utilization of glycogen and/or glucose. When accumulation is measured in terms of percentage, interestingly submaxillary glands showed higher

percentage (34.15) than that of parotid. This fact itself indicate that synthesis of mucin or mucopolysaccharides requires more use of glucose which is made available from glycogen. Whereas synthesis of molecules of  $\alpha$ -amylase a kind of protein require or demand comparatively less glucose utilization and hence percentage of increment or accumulation is less (30.23) in parotid and more in submaxillary gland i.e. 34.15 % of its value in normal condition.

Functions of each and every cell depends on structural as well as functional protein molecules. All the cells have their own genetically controlled machinery for synthesis of required protein molecules using the amino acids obtained from blood. Content of protein in any organ depend on several factors such as uptake of aminoacids from blood, rate of protein synthesis as well as degradation or hydrolysis and release of amino acids. Normally excess of aminoacids or proteins are not stored in any animal tissue. Total protein content of dry tissue weight is generally more than fifty percent. Total protein content of dry weight of tissue does not show much variation. However, different tissues contain different types of specific functional protein molecules. Generally protein content of fresh tissues show certain variation. It is more apparent as it depends on the levels of water, lipids, glycogen etc. and rate of synthesis of structural proteins as well as active or functional proteins in forms of enzymes etc.

Values of protein content per fresh weight of submaxillary and parotid glands are not much different, it is 17.24 mg% in submaxillary and 16.08 mg% in parotid gland. However, both the glands showed reduction in the protein content in diabetic condition. Submaxillary glands failed to show significant alteration but parotid gland showed small reduction in diabetic condition which is significant at the level  $p < 0.002$ . Anderson and Johnson, (1981), also observed reduced protein content of parotid salivary glands in diabetic condition of rats, but these authors induced diabetes by a single intraperitoneal dose of alloxan and studied after fourteen days. In the present study, we used multiple doses (5 doses) and sacrificed animals little earlier i.e. 11th day. It becomes clear that more or less similar response could be observed by multiple doses of alloxan little earlier also. Palla *et al.*, (1967) and Zebrowski and Brimmer (1978), have suggested that insulin insufficiency in rats leads to alterations in the level of secretory enzymes in the parotid gland of rat. Several studies have shown that both the level as well as specific types of proline rich proteins secreted in saliva can be altered by certain physiological conditions and

pharmacological factors (Fernandez and Carison, 1974; Robinovitch *et al.*, 1977; Muenzer *et al.*, 1979; Johnson, 1979, 1980). Even the action of mastication is known to represent physiological stimulation for the salivary glands (Johnson and Sereenbny, 1973), Tsunodu *et al.*, (1988), in their histochemical studies observed reduction or disappearance of glycoprotein from organelles such as rough endoplasmic reticulum, ribosomes, golgi cisternae and secretory granules of acinar cells of salivary glands of streptozotocin treated diabetic rats. Acinar cells of normal rats showed comparatively enough glycoproteins, it was thought that the amount of glycoprotein in acinar cells was decreased by the streptozotocin induced damage that occurred in golgi complex due to deficiency of insulin. The rate of synthesis of secretory proteins increase significantly in rat parotid glands after stimulated discharge of stored protein with isoproterenol (Kim *et al.*, 1989). Ekstrom *et al.*, (1989) observed correlation between amount of polyamines, rates of protein synthesis and tissue growth. Changes observed in the weight of salivary glands and activity of  $\alpha$ -amylase (Chapter I) indicate reduction in weight, slight decrease in protein content explain reduced protein synthesis in salivary glands of diabetic rats. Prosser and Hartman (1983) demonstrated elevated concentration of glucose in saliva of women during pregnancy and in non-lactating women preceding peak mucous days. These facts indicate the influence of hormones such as progesterone and estrogens on composition of saliva. Similarly, glucose in saliva of diabetic patients is also one of the important constituent, then one can even expect presence of aminoacids in saliva of diabetic animals. It is quite possible that uptake of aminoacid is not much affected. However, reduced protein synthesis would lead three possibilities : deamination, transamination or release of negligible amount of aminoacids along with its secretion saliva. Salivary glands are not known for active deamination or transamination, therefore secretion or release of aminoacids is quite possible. At this stage it is only a reliable predication and it require complete microanalysis of saliva of non diabetic (normal) and diabetic animals.

The results of the present study showed (table II) that the total lipid content of parotid gland is more than the submaxillary gland in normal condition. While both types of glands showed elevation in the total content of lipids in the diabetic condition. The increase in the total content of lipid is significant in both glands.

Lipogenesis is very common in visceral organs, tissues such as liver, adipose tissue, cardiac muscles and mammary glands (Harper, 1988). These organs or tissues are also involved in hydrolysis of lipids (Lipolysis). Lipids content of all these organs is comparatively high. Lipids are synthesized as a reserve food and many reports have suggested increased lipogenesis under influence of several factors including many hormones in different organs. Lipogenic effects of thyroxine (Goodridge *et al.*, 1974), gonadal hormone (Thapliyal *et al.*, 1975; Moore *et al.*, 1977; Patsch *et al.*, 1980; Umapathy and Rai, 1980), and insulin (Lardy *et al.*, 1965; Goodridge, 1973) have been clearly reported in hepatic cells (Woodside *et al.*, 1972). Popper and Schaffner (1957), have reported fatty liver in diabetic mellitus. Even Kupffer cells also become enlarged due to marked increase of lipids in diabetic condition (Volk, 1950; Popper and Schaffner 1957). Watanabe (1971) observed an increase in the compound lipids in liver of diabetic rats. In the salivary glands Wilborn and Schneyer, (1970) and Desai (1989) have reported that both the serous and mucous cells have lipogenic activities. It seems that these cells of salivary gland do synthesize little amount of lipids as a reserve food. Smooth muscle fibres and fibroblast of connective tissue in the certain peripheral part of acini also contribute to certain extent in this total lipid content of whole gland.

In general insulin do have stimulatory effect on lipogenesis through its stimulatory action on activities of malic enzyme (Lardy *et al.*, 1965; Goodridge, 1973). Even the stimulatory action of thyroxine on malic enzyme synthesis have been proved long back (Tepperman and Tepperman, 1964; Wise and Ball, 1964; Young 1968; Chandra bose and Bensudoun, 1971; Goodridge *et al.*, 1974). Thyroxin by activating the enzyme synthesis (Colton *et al.*, 1972) in liver, induces lipogenesis. In diabetic condition deficiency of insulin also lead to deficiency of thyroxin as Maraud *et al.*, (1965) reported that insulin stimulates the uptake of iodine by thyroid gland. Simultaneously lipids is also utilized. Utilization of lipids or its hydrolysis is also a characteristic feature of any normal active glandular cells. Main activity of serous type of cell is to synthesize protein,  $\alpha$ -amylase and mucous type cells synthesize mucin which contain several peptides, epidermal growth factor and many glycoproteins. Synthesis of all these macromolecules do require energy. It is possible that some amount of energy is made available from lipids. Thus active lipolytic activity is expected in salivary gland cells (Wilborn and Schneyer, 1970). Duncan (1951) found fatty deposits in the liver of patients of untreated and uncontrolled

diabetes mellitus and these deposits disappeared quickly when disease was controlled by insulin administration. The results of present work indicates that higher lipids content in both the salivary glands is most probably due to its accumulation. However, accumulation in terms of percentage of normal value is much higher i.e. 103.12% in submaxillary where as it is only 54.92% in parotid. These results indicate that lipid content of parotid glands is higher than that of submaxillary in normal rats. Both the types of glands showed increased value of lipid content which is probably due to accumulation resulted from reduced lipid utilization and not due to increased lipogenesis.

It could be concluded from the results of present work that rates of release or secretion of water along with saliva and uptake of water along with metabolites and material by the acinal cells run more or less parallel in submaxillary and parotid glands of normal and diabetic rats. Hence, both these glands failed to show significant alterations in their water content. Increased glycogen content of these glands of diabetic rats is due to accumulation resulted from its less metabolism or utilization or decreased synthesis of mucin and  $\alpha$ -amylase. It seems that synthesis of mucopolysaccharides require more glucose. Significant decline in protein content of parotid gland could be due to either reduced protein synthesis or release or secretion of amino acids along with secretion of parotid gland in diabetic condition. Increased lipid content of both the types of salivary glands could be due to accumulation resulted from reduced lipid utilisation in absence of insulin.