CHAPTER I

OBSERVATIONS ON LEVEL OF BLOOD GLUCOSE, BODY WEIGHT, RELATIVE WEIGHT AND ACTIVITY OF α-AMYLASE OF SUB-MAXILLARY AND PAROTID SALIVARY GLANDS OF NORMAL AND ALLOXAN TREATED DIABETIC MALE ALBINO RATS.

Diabetes mellitus usually defined as a chronic endocrine disorder of carbohydrate metabolism characterized by hyperglycemia and glycosuria. It is due to deficiency of insulin resulting from either insufficient supply or diminished effectiveness of beta-cells of islets of Langerhans (Joslin, 1959).

Glucose is an essential fuel for neurons, erythrocytes and muscular tissue. The higher centres of brain are especially sensitive to even short term deficiency of glucose. Since glucose enters most of cells via passive processes (down a steep concentration gradient), it is necessary to maintain adequate concentrations in the blood plasma. It is therefore not surprising that several hormones, epinephrine, glucagon, glucocorticoids and growth hormone etc. are charged with the responsibility of elevating blood sugar concentrations when the need arises. During periods of hypoglycemia glucose metabolism in nervous tissue is controlled by enzymatic machinery of the neurons and not by the surrounding concentrations of glucose (Sokoloff, 1974). The devastating effects of chronic hyperglycemia are related to the fact that glucose exists in the form of small water soluble molecules which are readily filtered by the glomeruli of the kidney and lost along with the urine as an abnormal constituent. Under normal conditions ultra filtered glucose is entirely reclaimed by the proximal tubules. But the reabsorption process requires energy (since work is done against a concentration gradient) and the rate of reabsorption is limited. Glycosuria need not to be associated with renal damage. Hyperglycemia then leads to glycosuria. Therefore there is a wastage of potential fuel, if there is no compensatory increase in glucose intake. In such conditions tissues will be broken down to supply carbohydrate requirements of neurons and erythrocytes (an early symptom of diabetes mellitus is the increased appetite accompanied by loss of body weight). Urinary excretion of glucose draws large volumes of water from the circulation (two additional symptoms of developing diabetes mellitus are the severe thirst and increased urine volume or polyuria). Content of the glucose in blood of the animals with diabetes exceeded 5 fold, its normal level in blood (Pushkina *et al.*, 1987). The blood sugar lowering effect of a 90% ethanol extract of Red sanders, *Ptercapus santalinus* (Wood) was studied by Nagaraju *et al.*, (1991); in fasted, fed, glucose loaded and streptozotocin diabetic models in albino rats. The material was found to be effective in lowering the glucose level. In alloxan diabetic rats the administration of diazoxide produced a more marked rise in the blood sugar than in normal sugar level of such diabetic animals (Staquet *et al.*, 1965).

Rat is a frequently used model of both exocrine secretion (Butcher and Putney, 1980; Baum *et al.*, 1983) and gerontological phenomenon (Goldberg and Roberts, 1981; Gray *et al.*, 1982; Goodrick *et al.*, 1983). Alloxan produces wide spread toxic effects on several organs including liver, kidney and pancreas (Lukens, 1948; Lazarus *et al.*, 1961). Most of these are reversible with time, but treated animals appear ill, eat poorly and lose the body weight for several days. It is likely that some animals are significantly starved due to drug induced illness and anorexia. There have been many studies reporting age related morphological changes in submandibular glands of human beings and rat with increased age (Andrew 1949; Bogart, 1967; Water house *et al.*, 1973; Scott, 1977). Changes in the several descriptive biochemical parameters in the submandibular glands of rat have also been noted (Bogart, 1970; Kuyatt and Baum, 1981). These include diminutions in RNA, protein, sialic acid and neutral sugar content in glands from more aged animals. Furthermore limited age related functional impairments have also been described, for example in response to the ß-adrenergic agonist iso-proterenol. The rat salivary glands are frequently utilized as model for *in vivo* study of exocrine protein and electrolyte secretion (Young and Schneyer, 1981). The effect of alloxan diabetes on the rat parotid gland may be similar, therefore to its effects on the exocrine pancreas in which there are non parallel changes in secretory enzyme levels (Soling and Unger, 1972). The principal physiologic role of these glands is the secretion of saliva and since submandibular saliva contains many factors critical to oral health (Mandel, 1980), such as epithelial growth factor (EGF) and nerve growth factor (NGF) (Hoshino and Lin, 1968, Thoenen and Barde, 1980, Gresik and Barka, 1983). Mammals usually lick their wounds and these factors may possibly aid in enhancing the healing of wounds (Harper, 1988). Byyny *et al.*, (1974) has suggested that the release of EGF in blood plasma and thus its level in plasma is controlled by sympathetic nervous system. It is well known fact that functions of all divisions of nervous system are also altered in diabetic condition. These neuronal alterations may affect elaboration content and release of EGF even in salivary glands in diabetic condition. Even growth of body or weight of body is directly or indirectly related to EGF content. As many factors are involved in regulating or controlling the structure and function of salivary glands, it was thought worthwhile to study these parameters in normal and diabetic conditions. If the descriptive changes in salivary glands noted above were to impair saliva production in animals, significant alterations in the health and function of the body would be a likely result. There are varied factors which cause the alterations in the metabolic activities of the animals and in turn various metabolic activities lead to the alterations in the body weight, weight of the salivary glands and the relative weight of the glands. For example due to some metabolic disorder there is a loss in the weight of the body, it may or may not lead to the reduction in the weight of the salivary glands, the relative weight of the gland is apparently increased. For example, sometimes the right gland is utilized more frequently than the left or vice-versa. Since the physiological role of saliva is the maintenance and protection of oral tissues involved in the critical functions of food acquisition and digestion, the general preservation of salivary function is of benefit to the animal. Very little information is available that compares total animal weight, salivary glands weight and relative weight of the glands.

Two major organs, the pancreas and salivary glands, provide the bulk of amylase. Human salivary amylase has been the subject of numerous studies, not only for its possible clinical significance but also for its function and properties as a purified enzyme. The availability of α-amylase in crystalline form from whole saliva through the studies of Meyer *et al.*, (1948), lead to the investigation of its physical and chemical characteristics (Bernfeld *et al.*, 1948; Muus, 1953; Mutzbauer and Schulz, 1965). However, since whole saliva is a complex mixture, it may conceivably contain several amylolytic components. It has in fact been reported that an oxygen-labile amylase is present in whole saliva (Bauer and Sredres, 1951). The actual separation of amylolytic components from whole saliva has been described by several investigators. Millin and Smith (1962), have reported the separation of three distinct amylases from whole saliva by a combination of gel filtration and chromatography on calcium phosphate gel columns. Norby (1964), employed agar gel electrophoresis to separate two, three or more zones of amylolytic activity from whole saliva. The biosynthesis and activities of rat pancreatic α-amylase are strongly affected by the composition of diet and by insulin level. Biosynthesis and the level of amylase in the pancreatic acinar cells and pancreatic juice increase 3-4 fold. When the percentage by weight of starch or glucose in the diet is raised from 20 diet p to 75 percent diet G (Reboud, 1962; Abdeljlil *et al.*, 1963; Abdeljlil and Desnuelle, 1964). Further more, the level of amylase is very low in the pancreatic acinar cells of alloxan diabetes rats and it is restored to normal or even higher than normal values by the regular administration of insulin (Abdeljlil *et al.*, 1965). Cross reactivity of salivary amylase with pancreatic amylase has been reported by Bernfeld *et al.*, (1950) and Takatsuka *et al.*, (1979). Since these two amylases are probably synthesized for the same purpose by different tissues in the same animal, it is of interest to see whether the regulatory mechanism for their biosynthesis is similar or different.

The purpose of present investigation is to study the alterations in blood glucose level, body weight, weight and relative weight of glands and the activity of α-amylase in two different types of salivary glands comparatively in normal and in insulin deficient condition of alloxan treated male albino rats.

Materials and Methods

Adult male albino rats, weighing 120 gms ± 30 gms were used as experimental animals. The rats were acclaimatized to laboratory conditions and maintained on a balanced diet and water *ad. libitum*. The animals were divided into three groups, each group comprising 8 to 10 rats.

1. Normal rats (untreated animals).

2. Control rats were given 0.9% saline (im). Total five doses were given on every alternate days. Each dose consisting of 0.5 ml of normal saline and animals were sacrificed on tenth day.

3. The experimental rats were kept starved for twenty four hours in the laboratory conditions and the intramuscular injections of 0.5 ml of alloxan monohydrate (30 mg/ ml in 0.9% saline). Total five injections were given on every alternate day to induce diabetic conditions in the rats. The animals were weighed and sacrificed on the eleventh day by cervical decapitation under mild ether anaesthesia in the morning

hours between 0900 hours to 1100 hrs. To find out the total weight of the body of rat in normal control and diabetic condition the rats were weighed on the spring balance just before the sacrifice in normal group and before treatment of saline and alloxan in control and experimental groups respectively. Thus the total weight of the body and the loss of body weight after the alloxan treatment during the period of ten days was determined and expressed in grams. The percentage loss of the weight and relative weight of submandibular and parotid salivary glands were also calculated. The samples of 0.1ml blood was collected from external jugular vein by micropipette for the estimation of glucose level in the blood. The level of blood glucose was estimated employing method described by Folin and Malmros (1929) and expressed as mg glucose/100 ml blood. The submandibular and parotid glands of both the sides were quickly excised freed from connective tissues and weighed on Mettler balance. The quantitative estimation of activity of α-amylase was carried out employing the method suggested by Sumner (1935). The activity of α-amylase is expressed in terms of mg maltose liberated in 3 minutes per mg protein in the homogenate at 37°C. Protein content of homogenate was estimated employing the method described by Lowry *et al.*, (1951).

Data on the level of blood glucose, body weight, loss of body weight, weight and relative weight of submaxillary and parotid salivary glands and the activity of α-amylase have been analyzed statistically and presented in the tables Ia, Ib and Figures Ia, Ib.

Results :

The results obtained are shown in tables Ia, Ib and Figures Ia, Ib, the total body weight of the animals in normal, control and diabetic rats recorded were 156.15 gms, 152.28 gms and 126.92 gms respectively. The loss in body wt of diabetic rats was 29.23 gms during the period of ten days of alloxan treatment. The data of present investigation indicate the significant loss in the weight of the body of diabetic animals when compared with their respective controls ($p < 0.001$). The loss in terms of percentage of body weight is 19.03%.

Table Ia

Total weight of body, weight and relative weight of sub-maxillary and parotid salivary glands of normal, control and diabetic male albino rats. Mean ± S.D.

Physiological condition animal	Weight of body[1]	Submaxillary gland		Parotid gland	
		Weight of gland[2]	Relative weight of gland[3]	Weight of Gland[2]	Relative weight of gland[3]
Normal	156.15 ± 24.67	182.14 ± 15.67	131.29 ± 17.35	187.42 ± 19.33	147.85 ± 13.12
Control	152.28 ± 20.14	174.14 ± 17.07	132.80 ± 18.21	188.28 ± 20.04	131.14 ± 17.35
Diabetic	126.92 ± 25.62	165.14 ± 18.77	137.00 ± 20.85	175.42 ± 17.84	113.63 ± 20.33
Significant (* P) at the level	P<0.001	NS	NS	NS	P < 0.02

1. Gm

2. Mg

3. Mg/100 G. Body wt.

*P values refer to differences between normal and diabetic conditions.
The students 't' test was used to analyse differences in means.

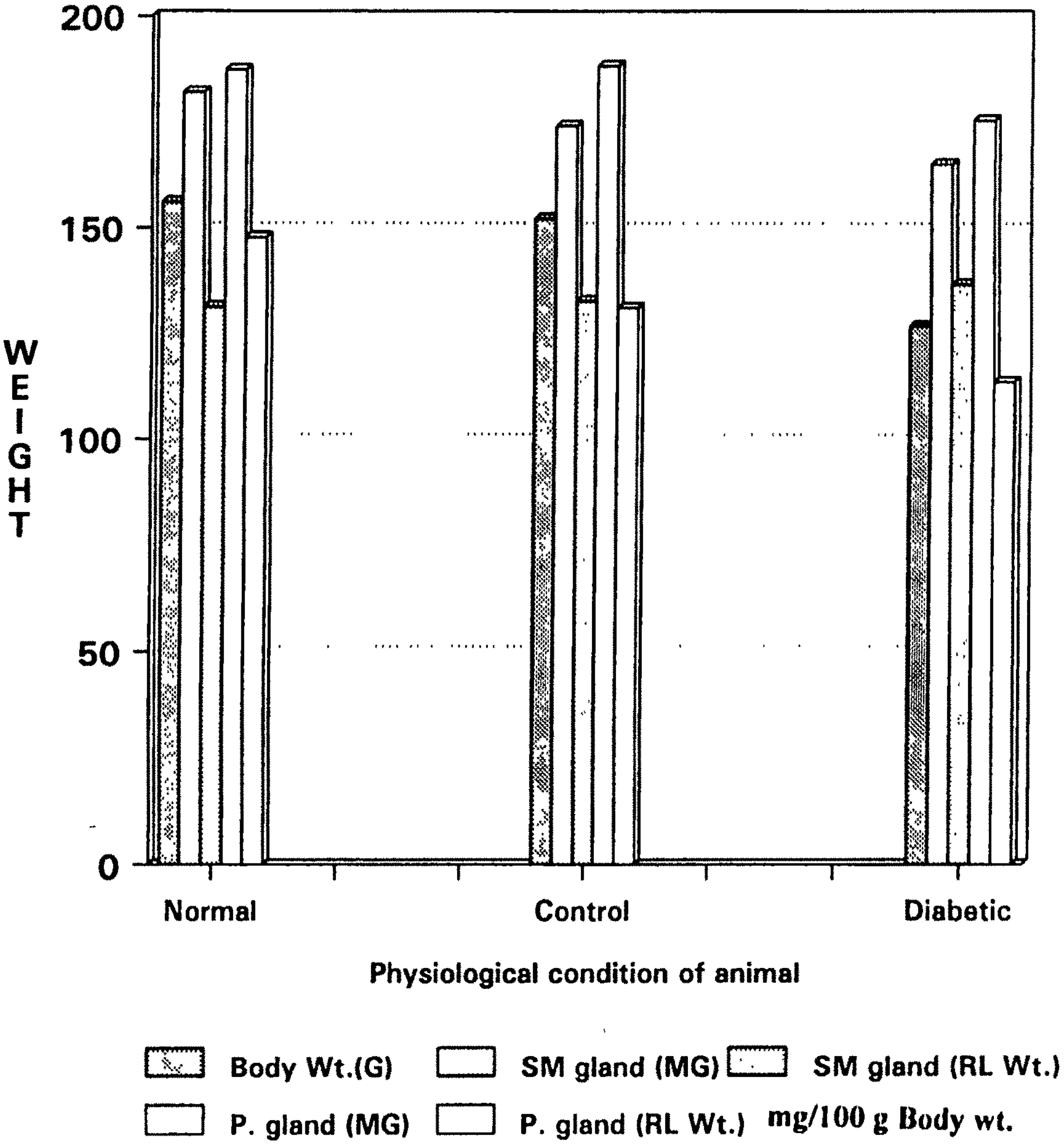

Fig. Ia. Graphic presentation of Total Body weight, weight and relative weight of submaxillary and parotid salivary glands of normal, control and diabetic male albino rats

Table I-b

Level of blood glucose and activity of a-amylase in submaxillary and parotid salivary glands of normal, control and diabetic male albino rats. Mean ± SD

Physiological condition of animal	Blood glucose level[1]	α-Amylase activity[2]	
		Submax. gland	parotid gland
Normal	127.56 ± 9.13	3.177 ± 0.070	5.890 ± 0.606
Control	123.04 ± 11.37	3.182 ± 0.040	5.552 ± 0.218
Diabetic	256.98 ± 15.34	2.813 ± 0.110	3.348 ± 0.379
Significant (* P) at the level	P < 0.001	P < 0.001	P < 0.001

1. mg glucose/100 ml blood

2. mg maltose formed/mg protein/3 minutes at 37oC

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

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

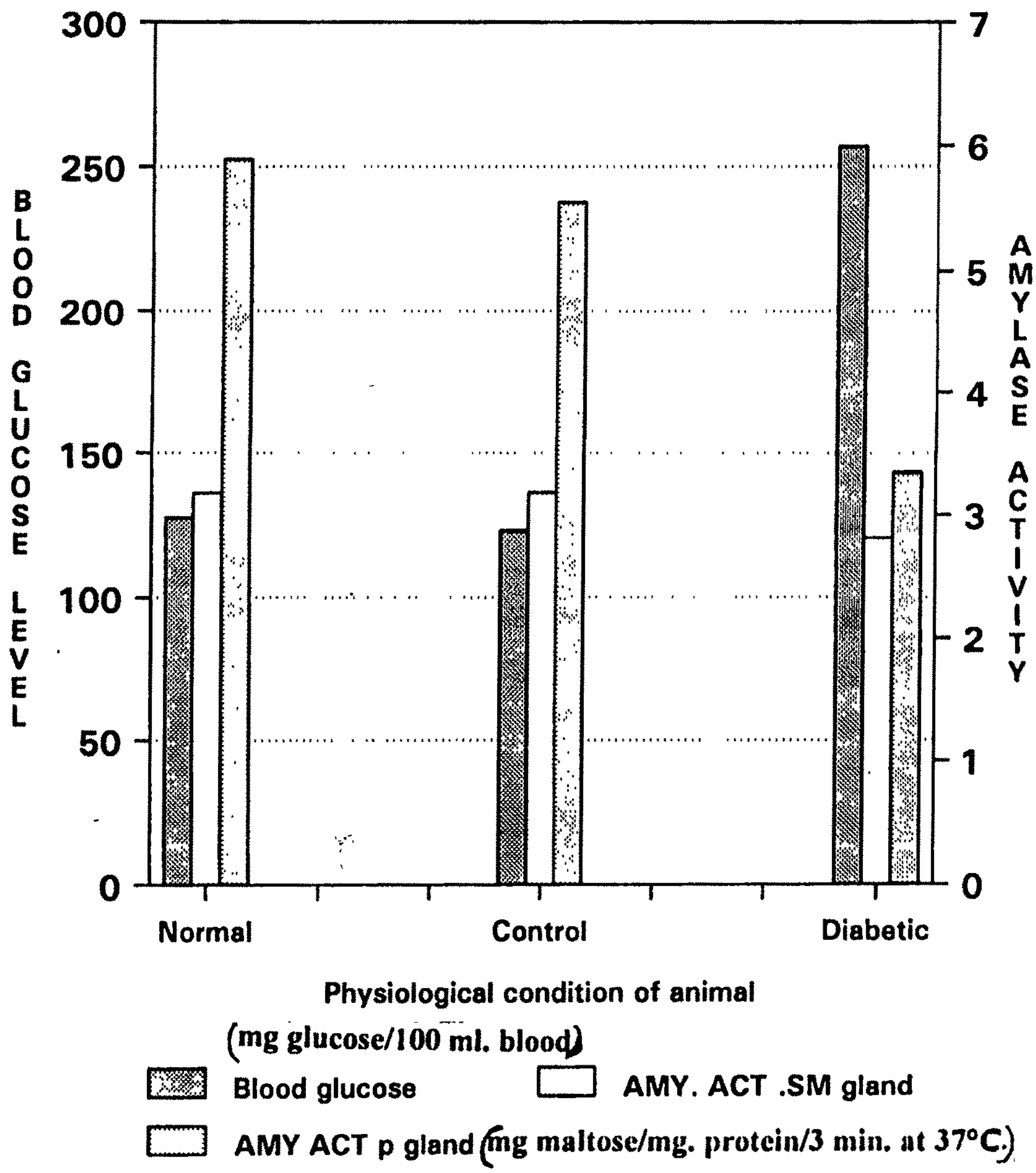

Fig. Ib. Graphic presentation of Blood Glucose Level and activity of α-amylase in submaxillary and parotid salivary glands of normal, control and diabetic male albino rats.

The weight of both the types of fresh salivary glands are shown in the table Ia and Figure Ia. The weight of submaxillary salivary glands of normal, control and diabetic rats are 182.14 mgs, 174.14 mgs and 165.14 mgs respectively, whereas weight of parotid glands in the same groups of animals are 187.42 mgs, 188.28 mgs and 175.42 mgs respectively. The submaxillary and parotid glands showed minor decrease in their weight in the diabetic rats. The reduction in the weight of gland is non-significant when compared with their respective weights in the normal rats. Thus both these glands showed similar trend of reduction in the weight of diabetic condition.

The relative weight of submaxillary salivary glands of normal, control and diabetic rats are 131.29, 132.80 and 137.00 mg/100 gm body wt. respectively and that of parotid glands in same groups of rats are 147.85, 131.14 and 113.63 mg/100 gm body weight respectively (Table Ia). The submaxillary gland showed slight and non significant increase in its relative weight in the diabetic rat. The relative weight of parotid gland showed moderate decrease in diabetic rats. The reduction in the relative weight of parotid gland of diabetic rats is significant when compared with respective values of normal rats ($p < 0.02$).

The data shown in the table Ib and Figure Ib indicate the value of blood glucose level in the groups of normal, control and diabetic rats. It is 127.56, 123.04 and 256.98 mg glucose/100 ml of blood respectively. Diabetic rats showed hyperglycemic condition, the blood glucose level become almost double i.e. increase is almost 100% in the diabetic rats in period of ten days of alloxan treatment. This change in blood glucose level is significant at the level of $p < 0.001$.

The activity of α-amylase in submaxillary and parotid salivary glands in the group of normal control and diabetic rats are expressed in terms of mg maltose formed/mg protein/3 minutes at 37°C temp (Table Ib and Fig. Ib). The values of activity of α-amylase of submaxillary glands of normal control and diabetic rats were 3.177, 3.182 and 2.813 respectively. The values of α-amylase activity of parotid gland of normal, control and diabetic rats were 5.890, 5.552 and 3.348 respectively. These results indicate that α-amylase activity is higher in the parotid gland than that of submaxillary gland in normal rats. Activity of α-amylase in both the glands showed reduction in diabetic condition which is statistically significant at the level of ($p < 0.001$) when compared with their respective normal values. Activity of α-amylase of parotid gland showed 43.15% reduction, whereas that of submaxillary gland only 11.45%. Thus activity of α-amylase of parotid gland showed higher reduction in diabetic condition.

Discussion

The results of present investigation have provided considerable information about the alterations in the body weight, weight and relative weight of the submaxillary and parotid salivary glands of the normal, control and diabetic rats. Body weight showed significant decrease or loss in diabetic condition of period after ten days of treatment. The loss of body weight is 29.23 gms and in terms of percentage it is 19.03%. The total weight of the fresh submaxillary and parotid salivary glands showed slight reduction in the diabetic condition when compared with their respective weight of glands of control group. The reduction in the fresh submaxillary gland weight is more (9.33%) then the fresh parotid gland (6.40%) in the duration of ten days of diabetic condition. The relative weight of these glands showed apparently different response. The fresh submaxillary gland showed non-significant higher value in the diabetic rat when compared with the value of normal rats, whereas the parotid gland showed the significant reduction in the relative weight in the diabetic condition when compared with respective normal and control values. The body weight of the diabetic rat when compared with that of non diabetic rats showed a reduction. Such loss of body weight in diabetic condition is reported by several investigators (Anderson *et al.*, 1981; Pillai *et al.*, 1989 and Suresh Kumar *et al.*, 1992). It has been reported that alloxan diabetes induces a significant enlargement of the small intestine of rat (Jervis *et al.*, 1966 and Nervi *et al.*, 1974). Similar enlargement of intestine was also observed in our experimental animals. This enlargement may be due to an enhancement of absorptive rates of hexoes (Schede *et al.*, 1971) and amino acids (Olsen *et al.*, 1970). The reduction in the body weight of diabetic animals may be due to reduced uptake of molecules of nutrients into the cells. Reduced anabolic activities are believed to be responsible for losses of fluid and electrolytes progress leads to the dehydration (Richard *et al.*, 1970). The dehydration was observed in our present experiment in the diabetic condition. The reduction in the weight of submaxillary and parotid glands in the diabetic condition was shown probably due to either reduced uptake of metabolites by the cells from blood or excessive loss of water along with less concentrated secretion on due to general degenerative changes. The relative weight of submaxillary and parotid salivary glands showed variation in their responses, the submaxillary gland showed a non-significant higher value in the diabetic state, while the parotid gland showed a significant reduction in the weight. This is probably due to more reduction in weight of parotid glands than that of body weight (Table Ia and Fig Ia).

Data of blood glucose are actually included here as a reliable index of diabetic condition. The results presented in table Ib and Fig Ib indicate that after ten days of treatment of alloxan, diabetic condition has been induced. Blood glucose level of diabetic rats is almost double than that of either groups of normal or control rats. Being a specific toxic agent to beta-cells of islets of Langerhans, alloxan administration induces deficiency of insulin. Deficiency of insulin leads to the diabetic condition. Several reports have shown that insulin stimulates activities glycogen synthetase (Bishop, 1970; Gold, 1970; Miller and Larner, 1973; Langdon and Curnow, 1983). In diabetic condition neuropathy like changes also takes place (Garrett and Thulin, 1975; Anderson *et al.*, 1984; Emmelin, 1987). Many investigators have also established neuroendocrine synergestic action between insulin and acetylcholine. Thus uptake of glucose by somatic cells is reduced. As a result glucose accumulates in the blood and hyperglycemic condition develop. There are several reports describing activities of several enzymes in salivary secretion and their relation with different hormone levels in the blood (Chretien, 1977; Berka, 1980). The parotid and submandibular glands of rats show that insufficiency of insulin induces alterations in the level of secretory enzymes (Palla *et al.*, 1967; Anderson *et al.*, 1981; Jaffa *et al.*, 1984). The decrease in activity of α-amylase in parotid gland content of mRNA in diabetic rats and reversal of these changes by insulin administration are similar to those changes that occur in the pancreas under the same conditions. However, the magnitude of these changes in parotid glands were much smaller than that of pancreas and the effect of insulin on α-amylase and m-RNA synthesis was not as immediate as in the latter gland (Kim *et al.*, 1990). Of the three salivary glands the parotid exhibited the highest amylase activity although both the glands showed reduction in fasting and diabetic conditions. Raynaud *et al.*, (1950) and Pillai *et al.*, (1989), showed the localization of amylase in the granular convoluted tubule (GCT) cells of submandibular gland. It's concentration is more in male GCT compared to that of females. Ekfors *et al.*, (1972), Cutler *et al.*, (1975) and Gresik *et al.*, (1979), showed the presence of these enzymes in GCT cells. This indicates that activities of α-amylase and trypsin in the submandibular glands depends upon GCT cells. GCT cells have been shown to be extremely sensitive to separate and combined actions of androgens, thyroid hormones and adrenocorticoids. Raynuad (1960) and Decamargo *et al.*, (1981) showed that in streptozotocin and alloxan induced diabetes, there was a change in structure of GCT of submandibular gland of male mouse where there was feminisation of GCT. Kallikrenin from a group of serineesteropeptidases which is present in GCT cells of rats and mice (Ekfors *et al.*, 1971 and Simson *et al.*, 1978). Jaffa *et al.*, (1984), reported a significant reduction in the activity and kallikrenin content of submandibular glands in alloxan and streptozotocin induced diabetic rats. Although previously reported studies (Palla *et al.*, 1967; Zabrowski *et al.*, 1978), suggest that insulin insufficiency in the rat leads to non parallel alterations in the levels of secretory enzymes in the parotid gland of rat the data have not been conclusive. The results of this study, however, clearly demonstrated the reduced activity of amylase in submandibular and parotid glands in diabetic condition. As reported by Anderson and Johnson (1981), the decrease in the activity of α-amylase was not due to displacement by lipid. The effect of diabetes on parotid gland of rat thus appeared to be similar to its effect on the exocrine pancreas (Palla *et al.*, 1967; Soling and Unger, 1972; Adler and Kern, 1975), the activity of amylase is reduced and the activities of chymotrypsin and lipase are increased in alloxan diabetes. Among the three salivary glands of male rats the parotid glands exhibited the highest amylase activity. The amylase activity in submaxillary and the parotid glands was significantly reduced in diabetic condition and parotid showed higher percentage of reduction. Pillai *et al.*,(1989) have reported less trypsin activity in all the glands in the alloxan diabetes rats than in the normal. The protein kinase C inhibitor, H-7 inhibited amylase secretion from rat parotid gland stimulated by PMA or the combination of phosphatidylserine and 1,2-diolein, the result supported the hypothesis that protein kinase C mediates amylase secretion in rat parotid glands (Shimomura *et al.*, 1988). Observations were made on amylase secretion from the rat parotid gland induced by parasympathetic nerve stimulation in vivo. Inspite of a low salivary amylase concentration, a large total amylase output equivalent to 36% of initial gland content was found on prolonged activation (5 H_2, 120 min). However, no decrease in gland amylase content was found, which indicates that the output of amylase was balanced simultaneously by synthesis also. The prolonged parasympathetically induced amylase secretion is dependent on activation of amylase synthesis and that the secretion is mainly of non granular origin. Parasympathetic nerve stimulation produced an even larger amylase output from sympathectomized glands. Even under these conditions gland amylase content was unchanged and the obvious degranulation was found when the glands were examined after stimulation. This is in marked contrast to the decrease in amylase content and to the degranulation which are found after sympathetic activation (Asking and Proctor, 1989).

Neuropathy like changes have been reported in visceral organs like liver and kidney of alloxan treated rats (Patel and Vaishnav, 1984). These investigators observed that uptake of glucose and the rate of glycogen synthesis in liver and kidney have been reduced due to absence of insulin as well as due to disturbed synergestic action of insulin and acetylcholine. Similarly one can expect that the uptake of glucose and other metabolites are also affected in the salivary gland cells. In the present investigation the absence of insulin and subnormal cholinergic activity of parasympathetic nerves are responsible for reduced activity of α-amylase in salivary glands of diabetic rats.

Oral glucose activates the amylase and glucose secretion by the salivary glands in human beings (Fekete *et al.*, 1990). Chilla *et al.* (1979), studied the influence of vegetative innervation of human submaxillary gland on human saliva and showed that a drop in the flow rate of saliva from the affected gland did not affect activity of amylase in the submaxillary secretion although total concentration of protein in saliva increased. However, these investigators indicated that protein secretion from the parotid gland occurred without rapid changes in the rate of secretory protein synthesis or transport. High flow rates, obtained by auriculo-temporal stimulation in rat have been reported to produce higher salivary amylase activity (Schneyer and Hall, 1965). Schneyer (1956) has also found similar results in humans. Ecosanoids evoke the release of amylase and increase ctytoplasmic calcium in rat parotid cells. Arachidonate and several of its metabolites increased amylase release and elevated cytoplasmic melittin, a stimulator of arachidonate mobilization and lyso-phosphatidylcholine also released amylase and elevated calcium. These results suggest that the metabolites of arachidonate may have an important role in amylase secretion (Snowdowne *et al.*, 1989). Experiment on male rats has shown that acute stress suppressed amylase and superoxide dismutase activity in the submandibular salivary glands. This influence of short repeated stresses weakened the inhibitory effect of the acute stress on the activity of the enzymes. A conclusion is drawn about the high sensibility of salivary glands to stress influences (Tarasenko *et al.*, 1990).

In summary, this study confirms that body weight of the male albino rats showed significant decrease on eleventh day of alloxan treatment. Submaxillary as well as parotid salivary glands showed non-significant decrease in weight in the diabetic condition. Relative weight of the parotid gland showed significant reduction in diabetic condition. Diabetic rats showed significant hyperglycemic condition. Activity of α-amylase also showed significant decrease in submaxillary as well as parotid salivary glands of diabetic rats.