GENERAL CONSIDERATIONS

Inspite of having vast amount of scientific information about the causes, pathophysiology as well as control measures of *Diabetes Mellitus*, it is still considered as an uncontrolled problem not only for developing countries but also for developed countries. Alterations in the structure as well as functions of most of the organs or tissues like liver, adipose tissue, kidney, muscular tissues etc. have been thoroughly studied in this endocrine disorder by many scientists. However, studies on structural and functional alterations of mammalian salivary glands have not been carried out in diabetic state.

The salivary glands are typical tubuloalveolar structure. On the basis of histological structure as well as biochemical nature of secretions, salivary glands are classified into three categories, serous type (parotid), mucous type (sublingual) and mixed type (submaxillary). Cells of serous and mucous acini are entirely different in their structure, metabolic activities, nature of secretion and even response to controlling factors. These glands are typical tubuloalveolar structure and composition of saliva is the final result of secretions of alveolar part of different salivary glands and reabsorption and/or secretion of water and electrolytes.

It is known since last several decades that functions of salivary glands is mainly controlled by two divisions of the autonomous nervous system. Stimulation of either branch will result in change in concentration and composition of secretion. The composition of this secretion depends on the type of innervating nerve stimulated (Langley, 1878; Carlson *et al.*, 1907; Kesztyus and Martin, 1973). When the sympathetic nerves are stimulated separately there is small secretion of protein rich, thick and viscous saliva and conversely, parasympathetic nerve stimulation results in a comparatively large volume of saliva of low protein concentration (Garrett and Thulin, 1975; Anderson *et al.*, 1984).

Norepinephrine released at the nerve endings causes protein secretion through ß-adrenergic receptors and water secretion apparently associated with the release

of potassium. These ß-receptors activate cAMP, well known second messenger while the ∝-receptors active another second messenger system i.e. Ca^{++}. ∝-adrenergic agonists cause release of plasma membrane bound Ca^{++} and influx of extracellular calcium ions, whereas ß-agonists do not.

In the body of highly evolved and complex animals quick and complicated functions of many glands have been controlled by more than one controlling system. In addition to neural control, mammalian salivary glands are known for showing response to certain hormones also. Pioneering work of Lacassagne and associates (1940 to 1970) has revealed sex related differences in these salivary glands of laboratory animals like rats, mice and several other mammals including human beings. Continuation of this investigation on the effects of sex hormones and ß-adrenergic agents on metabolism of submandibular salivary glands of rats has confirmed correlationship between gonadal hormones and salivary glands. Similarly, it is also a established fact that there is no single tissue or organ, whose function is totally independent from direct or indirect influence of insulin. Actually, food, blood glucose level, insulin level and exocrine as well as endocrine function of several organs of digestive system are interrelated.

Keeping in mind the main objectives of present work, two different types of salivary glands have been selected to study certain important parameters in normal health and in diabetic condition of male albino rats. Considering the occurrence of insulin receptors in almost all the organs, one could except influence of insulin on all the glands. Stimulation of uptake of glucose is the primary action of insulin. However, it is the type of glucokinase or hexokinase which determine the insulin dependent or insulin independent functioning of gland. The clear information about the occurrence and type of isoenzyme hexokinase in plasma membrane of serous type and mucous type of acinar cells is not available in literature. Whether serous and mucous types of cells differ in their response to insulin, what type of glucokinase isoenzymes are present in them, and whether metabolic activities and chemical composition of their secretion etc are influenced by hormones are not fully understood. In the present study an attempt has been made to understand the functional status of submaxillary and parotid glands in normal and diabetic conditions comparatively.

Insulin has direct effects on satiety and hunger centers in the brain, and thereby it regulates appetite, consumption and utilization of food in the body, affect the general health and body weight of an animal. In the present study, body weight of diabetic animal has indicated a significant reduction. This fact indicated that reduced uptake of nutrients and their utilization in the tissues of diabetic animals could be a reason. Hyperglycemic condition observed in the group of experimental (diabetic) male albino rats is a reliable index of diabetic condition. Like all other organs of digestive glands and different segments of alimentary canal these two types of salivary glands selected also showed alterations in response to deficiency or absence of insulin prevailing in the diabetic rats. The gross net weight of submaxillary and parotid gland showed reduction. However, short duration of ten days of diabetic condition failed to show significant reduction in their relative weight. From the results obtained in further investigation, it could be deduced that synthesis of different components of saliva was affected and thus secretory action of both types of salivary glands of diabetic rats showed alterations. This non-significant decline in the gross net weight of the glands indicated minor general degeneration in these glands, though it is very difficult to get correct picture about alterations in an individual organ or gland, hence the relative weight was also considered. Only the parotid gland showed significant decline in its relative weight. This fact indicated that parotid gland, containing only serous type of acini is much dependent on insulin, or more sensitive to insulin level, and this decline in the relative weight is by all probabilities due to the severe damage to the process of protein synthesis. _-amylase, the chief digestive enzyme of saliva is the main protein molecules in the secretion of parotid gland, whereas the submaxillary gland being a mixed type, failed to show such parallel decline in its relative weight as in the case of parotid.

Salivary glands are known for fast rate of their secretions. Like lacrimal glands under influence of catecholamines, salivary glands under influence of masticating action and autonomic nerves abruptly alter the rate of secretion and also chemical composition of saliva. From more or less steady state of water content of both the types of salivary glands in normal as well as diabetic condition it could be concluded that water being the main medium of transmembrane movement of materials, the rate of entry of water along with metabolites and minerals through plasma membrane of outer sides of acinar cells and rate of exit of water along with saliva run more or less parallel in normal and abnormal (diabetic) conditions.

Higher levels of glycogen in submaxillary and parotid glands of diabetic rats indicated that two main functions, i.e. synthesis and secretion of components of saliva, are affected or reduced in the salivary glands of diabetic rats. There could be more than one possible reasons for higher glycogen level in the salivary glands of diabetic rats. Acinar cells of these two salivary glands have hexokinase-I isoenzyme which is insensitive to plasma insulin level and thus glucose supply from blood is not affected. Reduced rate of synthesis of various components of mucin in mucous acini and similarly slow metabolism of glucose for synthesis of α-amylase and other peptides could be reason of accumulation of glycogen (chapter II). Comparatively higher percentage of accumulation in submaxillary gland is an index of higher and faster utilization, glucose and glycogen for synthesis of mucopolysaccharides and other components. This view could be supported from the observations of parotid gland. A comparatively higher value of glycogen in normal condition and less percentage of increment in diabetic condition indicated that glycogen content showed lesser response to the absence of insulin. Thus glycogen content of submaxillary gland showed greater response than that of parotid.

Much closer value of protein content of two types of salivary glands of normal rats is quite a striking feature; these two glands also showed more or less similar response to diabetic condition indicating that normal level of insulin is essential for synthesis and maintenance of structural as well as functional proteins. The role of insulin for stimulation of protein synthesis (Gelehrter and Tomkins, 1970; Reel *et al.*, 1970), ribosomal enzymes (Wool *et al.*, 1972) is well known. The reduction in the protein content (chapter II), and the parallel decline in activity of α-amylase (chapter I), in both types of salivary glands indicate that parotid gland is more insulin dependent and more sensitive to diabetic condition. Like glucose, certain amount of leakage or secretion of aminoacids along with saliva can also be expected in the absence of insulin.

Observations presented in this work also provide supportive evidence that even lipid contents of submaxillary and parotid glands is sensitive to the presence or absence of insulin. Like the important reserve metabolite glycogen, as described earlier, lipid content of both the salivary glands of diabetic rats showed higher level than that of normal condition. This kind of response is mostly due to reduced utilization of lipids rather than increased lipogenesis. Decline in the activities of

enzyme such as succinate dehydrogenase (SDH) in salivary glands of diabetic animals (chapter IV) gave an indication of subnormal mitochondrial activities. There could be two reasons : (1) direct inhibition of mitochondrial activities due to deficiency of insulin and (2) indirect inhibition through subnormal level of thyroxine. It seems that submaxillary glands use more lipid under influence of insulin and hence it showed comparatively more percentage of increment i.e. almost around 100%, than that of parotid which is only around 55%.

Considering the nucleus as the main controlling center of active cells of any gland or organ, the nucleic acids (DNA and RNA), have been estimated quantitatively in submaxillary and parotid salivary glands of normal as well as alloxan induced diabetic rats. Nuclear or chromosomal and cytoplasmic DNA has maintained its key position in regulating entire metabolic process of vital tissues or organs. Quick secretory actions of serous and mucous acini of these glands require high rate of transcription and translocation for synthesis of α-amylase enzyme molecules and mucopolysaccharides of mucin.

Insulin promotes positive nitrogen balance by stimulating uptake of aminoacids and net protein synthesis. It also accelerates the synthesis of RNA and biosynthetic activity of ribosomes. Severe insulin deficiency leads to negative nitrogen balance and excessive protein degradation in the diabetic state. Alterations observed in nucleic acids content did not coincide with related changes in the weight of these glands. DNA content showed unexpected small increment in both types of salivary glands although these glands showed decrease in net fresh weight (chapter I) which indicate that glands develop general degenerative changes, hence this small increase in DNA content in salivary glands of diabetic rats considered as an apparent rather than actual. Similar is the condition for RNA also. Leeb (1975), had claimed that mitotic figures are responsible for this small higher content of DNA. However this again require further more careful investigation before making final statement, because insulin normally stimulates division of cells in tissue culture.

Each cell generate the energy and use it for its various activities and for maintaining existence in a normal functional state. Insulin is known for stimulation of pyruvate dehydrogenase complex that produces acetyl Co-A neces-

sary for lipid synthesis as well for further metabolism through TCA cycle (Baxter and Coore, 1978; Hutson *et al.*, 1978). The stimulatory action of insulin on mitochondrial function is well known since last several years. Insulin enhances supply of pyruvate to mitochondria. By stimulating uptake of glucose through activation of hexokinase and by stimulation of glycolysis. As an index of TCA cycle activation, activity of its key enzyme, succinate dehydrogenase (SDH) has been estimated in these glands of normal and chemically induced diabetic rats. Very high and significant decline in the activity of SDH in the glands of diabetic rats proved that the deficiency of insulin has affected the mitochondrial activity. Inhibition observed in diabetic condition is more or less equal i.e. around 50% in both the glands. However, in normal condition submaxillary glands have comparatively higher SDH activity than that of parotid in presence of insulin.

On the other hand expenditure of energy for various cellular activities could be judged by measuring the rate of hydrolysis of ATP. Hence activity of ATPase has been studied quantitatively in these salivary glands of normal and diabetic rats. Activity of ATPase in salivary glands also showed similar response to the deficiency of insulin just as that observed in the activity of SDH. It could be concluded that a reduced or subnormal metabolic activities in the diabetic condition reduced the demand of energy which was reflected on the activities of ATPase. Both the types of salivary glands of diabetic rats showed reduction which more or less identified around 16 to 18 % of the control value (chapter IV). Assuming the possibility of existence of insulin-insensitive hexokinase - I isoenzyme in these glands, one should not also expect any alterations in membrane bound Na^+-K^+-ATPase involved in the uptake of metabolites by acinar cells from blood. However, reduced or subnormal mitochondrial activity and disturbed reabsorption process by the cells of tubular system of these glands could be considered as possible events responsible for showing declined activities of ATPase in diabetic condition. Changes in the chemical composition and nature of saliva of uncontrolled or untreated diabetic patients provide support to this assumption of alterations in reabsorption phase in the process of saliva formation.

Now it is proved beyond any doubt that ascorbic acid (AA) is a vitamin for only few animals including human being but is very significant biomolecule with several important functions. Considering varied and multiple roles in many organs in several

physiological and endocrine conditions it was thought desirable to carry out quantitative assay of AA in these glands of normal and diabetic rats. Simultaneously, cholesterol level also has been quantified in these salivary glands. The results of this study indicated that submaxillary and parotid glands have maintained more or less same status with reference to their contents of AA and cholesterol in normal condition (chapter V). Higher levels of AA in both the types of salivary glands of diabetic rats suggest its accumulation due to its less utilization in acinar cells in which many activities operate with a minimized rate in the absence of insulin. Higher percentage of increment due to comparatively more accumulation in submaxillary gland indicate its greater involvement in synthesis of mucopolysaccharides in submaxillary gland. Interestingly, non-significant increment of AA in parotid gland of diabetic rats does not only support above mentioned statement but also indicates that AA is less involved in the function of cells of serous acini in parotid gland. More or less parallel increment in values of cholesterol level in these two types of salivary glands is the indication of either diversion of other metabolites for hypercholesterologenesis or accumulation due to reduced utilization. It would be right and necessary to draw attention that hypercholesterolemia of diabetic condition is generally the result of hypercholesterologenesis in liver, intestine etc. due to deficiency of insulin.

It is difficult to explain a definite involvement of the non-specific acid and alkaline phosphatases by its just quantitative assay in a complete gland and exact reasons of any variation in the activities in these tubuloalveolar glands in normal and diabetic conditions. It becomes further complicated in submaxillary glands where two different kinds of acini (serous and mucous types) are present. However, a preliminary attempt has been made to observe any variations in the quantitative levels of activities of these enzyme. Submaxillary salivary glands exhibit comparatively higher activities of both the phosphatases in normal condition. Increase in the activities of acid phosphatase of parotid gland of diabetic rats was believed to be due to the activation of glucose-6-phosphatase which also have an optimum pH in the acidic range and a similar response to the insulin deficient condition. An occurrence of glucose in saliva of diabetic patients could be due to its abnormal release or secretion by acinar cells under influence of active glucose-6-phosphatase and/or failure of tubular structure as well to reabsorb it in the absence of insulin. It could be believed that hyperactive lysosomal activity in diabetic condition might

be responsible to a certain extent for this higher activity of acid phosphatase. Alkaline phosphatase of submaxillary gland showed much higher activity than that of parotid in normal rats in presence of insulin. It seems that comparatively higher activity of alkaline phosphatase in the submaxillary gland could be due to its participation in alveolar secretion and tubular reabsorption in normal condition. Its decreased value in diabetic conditions coincide with accumulation of glycogen as observed by Moog (1965). On the other hand parotid gland did not register any decline in alkaline phosphatase activity in diabetic condition also. This suggest that alkaline phosphatase is not much involved in parotid gland.

From the present preliminary study it is difficult to conclude exactly (1) whether secretory function of acini or reabsorptive function of tubular part of these glands are affected or altered in diabetic condition and (2) whether uptake of metabolites from peripheral part of acini or secretory function of saliva on the inner side of acinar cells are affected. In case of submaxillary gland it was much difficult to conclude for any changes whether occurred in serous or mucous acini. The study, of salivary glands, however particularly in diabetic condition, indicate the requirement of further detailed investigations from various other angles. The present findings also open up new fields for further studies which may lead to the establishment of a definite relationship between insulin level of plasma and micro composition of saliva and in future this information will help in possible diagnostic application of saliva in monitoring diabetic condition.