# INTRODUCTION

The term 'Diabetes' is derived from a Greek word meaning 'Siphon' and therefore refers to the large urine volume, 'mellitus' (honey), denotes the high sugar content. 'Diabetes mellitus' is therefore 'sugar diabetes'. The first description of diabetes is available from Charak sanhita (3000 B.C.). It has been classified in twenty varieties. The etiological factors described are :

(1) Rich calorie diet and sedentary habits.

(2) Hereditary factors like genetic defect or chromosomal abnormality.

(3) Obstruction in the flow of blood in the vessels supplying the viscera.

Characteristics of disease have been described to be loss of energy and sweatness. These patients have been divided in to two groups i.e. obese (sthoola) with good body built and energetic, second group being emaciated and weak. They have been described to be crazy for food, not interested for physical work, inactive and have the habit of taking the excessive fried food items. Sushrut Sanhita (2000 B.C.) had described that about twenty types are ultimately converted into diabetes mellitus which is incurable in many cases who have complications like carbunches. In Asthanga Sangraha (600 A.D.), diabetes mellitus has been described to be a disease of metabolic disorders. In Astanga Hridya (700 A.D.) diabetes mellitus means presence of sugar in the urine with increased blood sugar level, cough, fever coma, diarrhoea and cardiac trouble (Ojha *et al.*, 1973).

Actually, diabetes is not a disease but an endocrine disorder. The natural history of diabetes mellitus can be arbitrarily divided into four stages based on the presence or absence of abnormal carbohydrate metabolism. Overt diabetes is the most advanced stage, characterized by elevated fasting blood glucose concentration and other classical symptoms. This stage is divided into ketotic and non-ketotic forms. Preceding overt diabetes is the latent or chemical diabetic stage without definite symptoms of diabetes but demonstrable abnormality or oral or intravenous glucose tolerance. Subclinical diabetes is an earlier stage, when glucose tolerance is abnormal only with stress, such as pregnancy or the administration of cortisone. The

earliest stage of prediabetes extends from conception until the first demonstrable abnormality in glucose tolerance. In groups of presumed prediabetic individuals delayed and/or decreased plasma insulin response to glucose has been noted. Progression of the diabetes may not occur, may occur very slowly or very rapidly and regression top and earlier stage of abnormality may also occur (Stefan, 1973).

There are two common types of diabetes mellitus, type I and type II. Type I diabetes which is called Juvenile diabetes or insulin dependent diabetes mellitus (IDDM), which precipitates very rarely but suddenly among small children due to the quick disfunction of beta-cells of islets of Langerhans. Beta-cells stop synthesizing and secreting insulin either due to absolute destruction of beta cells by viruses or due to mutation in genetic complex. Type II, maturity onset diabetes (MOD), which is very common in adults. It develops after maturity. It is also called non-insulin dependent diabetes mellitus (NIDDM). Deficiency of insulin and/or insulin resistance are the main causes, the deficiency of insulin can be corrected by certain beta cells stimulatory drugs, such as Tolbutamide, Chlorpropamide, tolazamide, and glibenclamide. These oral hypoglycemic compounds are sulphonylurea compounds. These drugs act by stimulating the production of insulin by the Beta cells of islets of Langerhans. They also have a secondary effect in reducing hepatic glucose output. The beta cells secrete insulin also in response to other stimuli, notably certain amino acids and the drug orinase. Because of this property, orinase is used as an 'oral insulin' in the treatment of certain kinds of diabetes.

Actually, survival and normal functioning of each and every tissue or cell of body depends on the supply of nutrients from body fluids as nutrients are only the source for generating energy required for synthesis of structural and functional biomolecules. Although glucose is the main physiological regulator of insulin secretion, amino acids as well as low level of glucagon released after a meal can also play a role (Ashcroft, 1980; Cooperstein *et al.*, 1981; Wollheim *et al.*, 1981; Malaisse *et al.*, 1981).

Insulin is a hormone which is essential for almost all organs. Existence of insulin receptors on plasma membrane of different types of cells/tissues has been suggested by many research workers (Vigneri *et al.*, 1978; Holman *et al*, 1983). Insulin affect

the functions of cells of almost all organs of body directly or indirectly. Studies on structural and functional changes have been predominantly carried out on most of the tissues or organs like liver (Schein *et al.*, 1971; Nervi *et al.*, 1974; Eleuterio *et al*, 1990), kidney (Scalera *et al.*, 1981; Murer *et al.*, 1986; Levy *et al*, 1990;) cardiac muscles (Wharton *et al.*, 1965; Dang *et al.*, 1988), muscular tissue (Kruszynka *et al*, 1988), adipose tissue (Fantus *et al.*, 1987) and blood (Takahashi *et al.*, 1986; Axelrod *et al.*, 1986) in diabetic condition. Very few studies are reported on lacrimal and salivary glands (Dale and Laidlaw, 1912; Trendelenburg, 1954). However, since last two decades few investigators have focussed their attention to study the effects of alloxan diabetes and insulin on rat salivary glands (Palla *et al.*, 1967; Liu and Lin, 1969a, 1969b; Szymczyk *et al.*, 1971; Murakami, 1974; Zebrowski and Brimmer, 1978; Anderson and Shapiro, 1979, 1980).

Insulin is thought to be necessary for normal salivary gland's growth and function in the rat (Liu and Lin, 1969a; Anderson and Shapiro, 1980). The insulin secretion by the beta-cells of the islets of Langerhans is regulated through the mediation of autonomous nervous system (Woods and Porte, 1974), these reflexes are:

(1) in response to changes in the blood glucose level.

(2) in response to the stimulations related to food (smell, taste etc.) and

(3) conditioned through learning.

As cholinergic and adrenergic nerve fibres innervate the islets both are implicated in the control of hormone secretion from islets. Stimulation of vagus nerve generally elicit secretion of insulin. Chieri *et al.*, (1975), showed that in dogs glucose load to the brain induces pancreatic insulin secretion mediated partially by the vagus nerves, conversely the rate of insulin secretion can be inhibited by direct neural input to the pancreas and this inhibition is mediated by alpha adrenergic receptors (Miller, 1975). The control of insulin release from the islets is normally the function of glucoregulator center of the brain which is sensitive to both glucose load and insulin (Szabo and Szabo, 1975a, 1975b). Insulin lowers the blood sugar, the changes which seem to account for the fall in blood sugar may be summarized as follows :

(1) Glucose is taken up from the body fluids and (a) increased deposition of glycogen in the liver and muscles. (b) Increased rate of conversion of glucose in to fatty acids.

(c) Increased rate of oxidation of glucose through glycolysis and TCA cycle.

(2) The entry of glucose into body fluids is reduced by : (a) Depression of gluconeogenesis, the formation of glucose from non carbohydrate sources, mainly protein. (b) Depressed rate of conversion of glycogen into glucose in the liver.

Cori (1946), believed that insulin released the enzyme hexokinase from inhibitory effects exerted by pituitary and adrenal gland so that the phosphorylation of glucose could proceed normally. Metabolic effects of insulin deficiency are accelerated glycogenolysis in liver, increased gluconeogenesis, decreased entrance of glucose in to peripheral tissues, hyperglycemia, glycosuria, accelerated oxidation of fatty acids in the liver, over production of ketone bodies ketonuria, decreased fatty acid synthesis decreased protein synthesis in the peripheral tissues, increased urea formation and its excretion.

In addition to performing functions such as protection and absorption, the cells of an epithelial sheet may elaborate secretory materials also. Epithelial cells adapted specifically for secretion constitute the glands. In cyclostomes, there is a large so called "Salivary gland" of unknown function opening in to mouth on either side below the tongue, with this exception, glands are absent in the mouths of aquatic, ichthyopsida. With the assumption of pulmonate respiration and more terrestrial habits the mouth is no longer constantly bathed with water and glands appear, increasing in numbers and complexity in the higher forms of terrestrial vertebrates. The secretion of these glands helps in moistening the food and not frequently it is adhesive and is used in capturing the prey. In the terrestrial amphibians, reptiles like snakes and lizards, there are labial glands opening at the bases of the teeth and an intermaxillary or internasal glands in the septum between the nasal cavities as well as palatal glands near the Choanae (the internasal gland is lacking in the Caecilians). Many reptiles also have a sublingual gland on either side. In many snakes a pair of the labial glands are greatly developed and have migrated into the zygomatic ligament, where they have become modified into well known poison glands. The ducts of these poison glands are connected with the poison fangs. In the only known

poisonous lizard Gila monster (*Heloderma*) the sublingual glands furnish the poison. Poorly developed oral glands in the sea turtles and the crocodiles indicate secondary degeneration related to their affinity for aquatic life. Birds lack the labial and internasal glands but they have numerous other glands opening separately in to the roof of the mouth, as well as anterior and posterior sublinguals and frequently an 'angle gland' at the angle of mouth, which may be at last remnant of the labial glands of the other sauropsida. It is in mammals that true salivary glands appear. Besides there are numerous smaller glands (labials, buccals, linguals, palatines) embedded in the mucous membrane and opening separately into the mammalian mouth. These salivary glands are usually in the neighbourhood of the mouth, but one or many of them may be carried back and thus located into the neck, but in all cases the homologies can be judged by the position of openings of their ducts in the mouth. The gross morphology of salivary glands varies between species to species. The salivary glands include the submaxillary and sublingual of the lower mammalian groups and in addition the parotid gland, apparently a development within the class. The parotid gland is conspicuous in both lateral and ventral views. It is not a compact structure and runs well up behind the ear and down over the ventro-lateral side in the neck where it lies along the posterior facial vein and is in contact with the lymph glands of the neck. It's posterior extremity reaches the shoulder and covers the outer half of the clavicle. The exorbital lacrimal gland is closely associated with its anterior border and covers the beginning of the parotid duct (Stenson's duct). The duct formed by the union of three principal branches, crosses the masseter muscle parallel with the buccal and mandibular branches of the facial nerve and opens opposite the molar teeth. The two large submandibular or submaxillary glands are the most prominent structures of the ventral cervical region. They are in contact along the mid-ventral line from the level of the hyoid-almost to the manubrium. The submaxillary duct also known as Wharton's duct, closely applied to the latero-anterior surface of the submaxillary is a gland which appears at first to be a lobe of the submaxillary but which was found by Huntington and Schulte (1912), to be the major sublingual. Loewenthal (1912), speaks of this as the gland retrolingualis and calls it a second or accessory submaxillary. Huntington and Schulte state that "In rodents submaxillary and major sublingual ducts run beneath the mylohyoid muscle and open by separate parafrenular openings beneath the floor of the mouth at the side of the frenulum linguae on the plica-sublingualis. Minor sublingual glands open by several ducts on the lesser sublingual (Ravinian) ridge. Their description of the gland of muskrat, as a compact mass in the alveolingual distinct covering lateral aspect of the

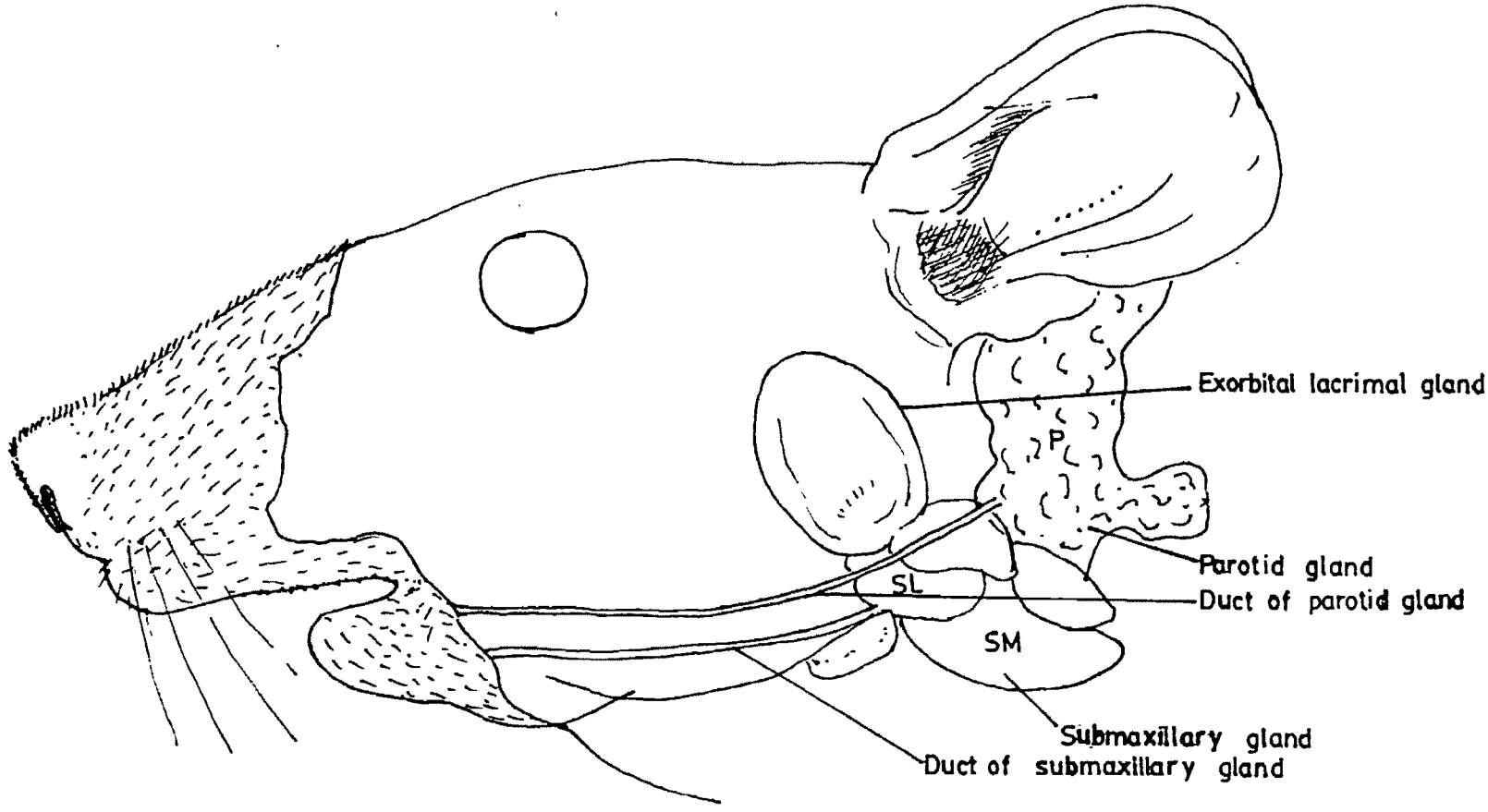



**Fig: Diagramatic presentation of Lateral view of Viscera of neck of Albino Rat showing relative position of submaxillary and parotid salivary glands and their ducts. (SL-major sublingual gland).**

submaxillary and greater sublingual ducts", is also true for the rat. This gland lies in contact with the mucous membrane of the floor of the mouth just in front of the lingual nerve.

The salivary glands of mammals are typical tubuloalveolar structures. The cells lining the alveoli are known as end piece cells (Young and Van Lannep, 1978). The salivary glands consists of three cell types, serous, mucous and seromucinous cells. The parotid, the largest of the major salivary glands, is compound tubuloalveolar, and in man, most domestic animals and rodents it is entirely serous. The cells of this gland have small granules containing amylase and some amount of mucopolysaccharides. A few mucous cells are present in the parotid glands of carnivorous young puppies and lambs. The sublingual, a composite of glands of variable sizes, is a mixed compound tubuloalveolar gland and cells contain droplets of mucous and acid mucopolysaccharides. The submandibular gland like the parotid, is a compound tabuloalveolar, mixed gland with seromucinous cells containing both acidic and neutral mucopolysaccharides. In rodents the gland is primarily serous. The mucous tubules usually show serous crescents or demilunes at their blind ends, small channels, the intercellular secretory canaliculi pass between the mucous cells and extend between the serous cells the demilune. The system of ducts of salivary glands comprises intercalated, striated or granular and excretory ducts. The structural arrangement of acini is compatible with function of primary salivary secretions. The latter, then known to be modified by processes of absorption and secretion during its passage through the various regions of the system of ducts. The phenomenon has been shown to be functionally comparable to that occurring in nephrons, at least as far as the flux of electrolytes and water (Winston *et al.*, 1988), is concerned. The major salivary glands of rats and mice include the paired parotid, submandibular and sublingual glands. The parotid gland is serous through-out development (Redman and Sreebny, 1970; 1971). The submandibular gland is mixed containing both serous demilunes and mucous acinar cells. In sublingual gland, the mucous and serous cells appear to arise separately and coincedentally at 18 days post conception and they constitute distinct cell populations from the time of their inception (Redman and Ball, 1978). Submaxillary gland of adult rat and mouse contains mucin producing acinar cells as well as granular convoluted tubule cells, which are specialized secretory cell type that develops postnatally (Cutler and Chaudhry, 1975). The parotid secretion, has characteristic clear and watery character; serous secretion

being much less viscous, but richer in amylase; while that of sublingual gland is thick opalescent, sticky and rich in mucin. The submaxillary gland contains alveoli made up of mucous cells and other made up of serous cells. The two types may be seen side by side and in about equal numbers. The mixed saliva emanating from all these glands, is viscous colourless and opalescent fluid with variety of compounds including water comes from the blood by way of tissue fluid, inorganic ions, organic components like glycoprotein, mucin, enzyme ptyalin, aminoacids, urea, lipids, citrates etc. Some heavy metallic ions like lead and mercury may also appear in saliva occasionally. Glycoproteins in saliva include neutral as well as sulfated mucins and those containing only sialic acid components. These give saliva its viscosity and lubricating property. Blood group substances represent an important component of salivary glycoproteins. The secretion of these cells is believed to reach lumen of the alveolus by means of small canaliculi between the mucous cells lining the alveolus.

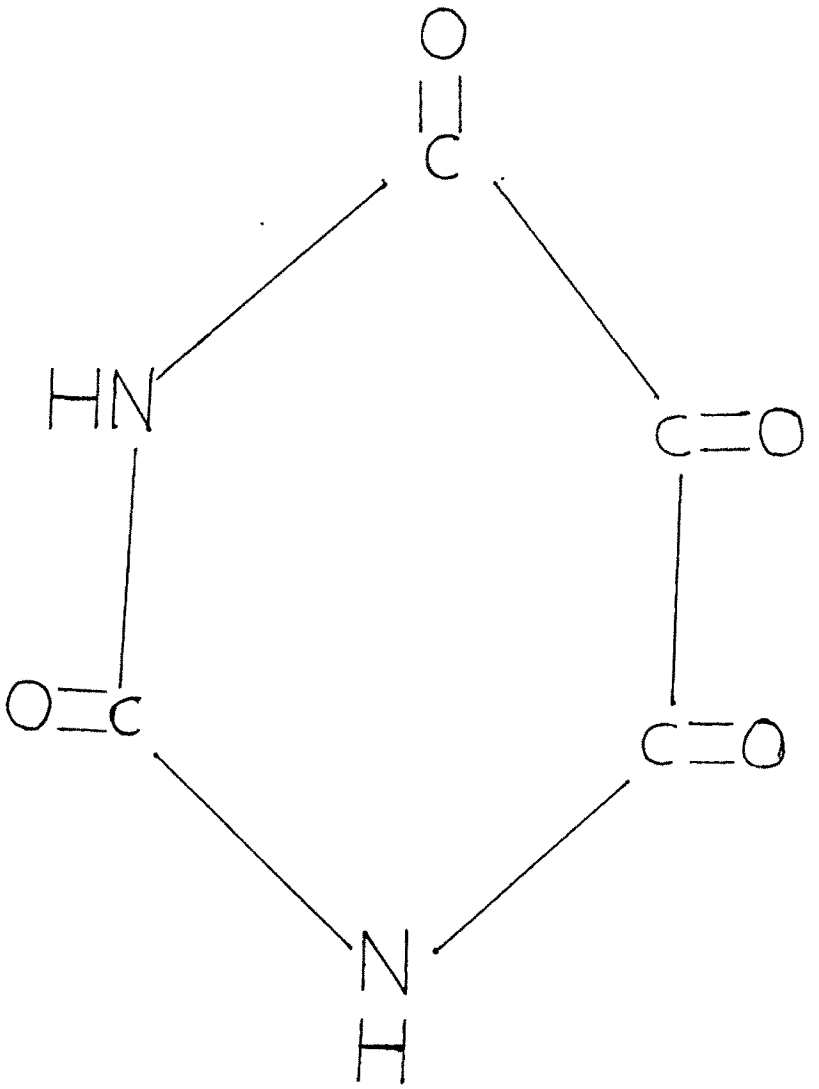
It is a well established fact that salivary secretion is controlled by nervous system. Russian scientist Pavlov (1910), has observed control of salivary secretion by conditioned reflex action. Now it is also known that salivary glands elaborate epithelial growth factor (EGF) and nerve growth factor (NGF) (Hosino and Lin, 1968; Gresik and Barka, 1983). According to Byyne *et al.*,(1974), the release of EGF in blood plasma is controlled by sympathetic nervous system. By all possibilities one can assume that trace of these growth factors i.e. EGF and NGF are also secreted out along with saliva. It is a common observation, that many animals have a habit of licking their wounds and this habit enhances the wound healing. It is possible that these growth factors stimulate proliferations of epithelium and innervation to this newly developed tissue.

The innervations of salivary glands comprises parasympathetic and sympathetic nerve fibres (Best and Taylor, 1985). In cats, parasympathetic stimulation produces copious watery secretion of saliva, whereas sympathetic stimulation produces viscous saliva containing greater proportion of solids from the submandibular gland (Bell, Davidson and Smith, 1957). The sympathetic secretory nerve fibres of the salivary glands in rat except the sublingual act via the mediation of catecholamine receptors viz α- and ß-types (Emmelin *et al.*, 1965). The functioning of the glands depends on the integrity of both adrenergic as well as cholinergic nerves (Bloom *et al.*, 1981). It is known that salivary glands of mammals are influenced by adrenergic

as well as noradrenergic transmitters (Strombald and Nickerson, 1961; Assarson and Emmelin, 1964; Nordenfelt, 1964; Mangos *et al.*, 1975a and b; Garrett, 1965 and 1966; Schneyer and Hall, 1965; Case *et al.*, 1980; Ekstrom, 1980). Chronic administration of ß-adrenergic agonist, isoproterenol has been known to produce hypertrophic and hyperplasic enlargement of submandibular glands in rats (Schneyer, 1962). In rats and mice enlargement of salivary glands comparable to human sialadensis, can be produced experimentally by administering analogs of epinephrine viz. isoproterenol and aludrine (Seifert, 1966). It was shown by Abe *et al.*, (1980), that parotid glands of rat respond to epinephrine in a dose dependent fashion. Schneyer and Hall, (1965), have reported that use of adrenergic agents or stimulation of sympathetic fibres to submaxillary or parotid glands of rat causes maximal flow of saliva. However, saliva evoked by action of adrenergic mediation is reported to be generally higher in organic content and certain inorganic salts (Mc Clanahan and Amberson, 1935; Langstroth *et al.*, 1938; Schneyer and Schneyer, 1961; Yoshida *et al.*, 1967). Bullard and Delsuc (1953), based on histological studies have observed that submandibular glands of female mice assume appearance of male type after receiving testosterone.

Several studies on the effects of alloxan induced diabetes and insulin on the rat salivary glands have been reported in literature (Palla *et al.*, 1967; Liu and Lin 1969a, 1969b, Szmyczyk and Brimmer, 1978; Anderson and Shapiro, 1979, 1980). Insulin is thought to be a required hormone for normal growth and function of submandibular gland in the rat (Liu and Lin, 1969a; Anderson and Shapiro, 1980). Activity of amylase in the parotid gland appear to be reduced in diabetics (Palla *et al.*, 1967; Zebrowski and Brimmer, 1978) and studies of 14C valine incorporation suggest that only amylase synthesis and not protein synthesis in general is affected (Palla *et al*, 1967). The effect of alloxan diabetes on the rat parotid gland may be similar. Therefore to its effect on the exocrine pancreas in which there are non-parallel changes in secretory enzyme levels (Soling and Unger, 1972).

Experimental insulin deficiency more commonly achieved in warm blooded animals by administration of agents which selectively destroy the beta-cells of pancreas. Alloxan has been useful for selectively destroying insulin secreting cells while leaving glucagon secreting cells of the islets intact. In the present study alloxan was used to induce diabetes in rats, because of its ease of application and availability,



**MOLECULAR STRUCTURE OF ALLOXAN**

**Alloxan ($C_4 H_2 N_2 O_4 H_2O$), a derivative of pyrimidine that produces experimental diabetes in animals by destroying Beta-cells of Islets of Langerhans.**

the diabetogenic action of alloxan has been known for more than thirty years, since Dunn *et al.*,(1943), noted this effect while studying the crush syndrome in rabbits. The exact mechanism by which alloxan produce selective destruction of the beta-cell is unknown, though various hypothesis have been proposed, such as chelation of zinc, alpha aminoacid deamination and decarboxylation (the stecker phenomena), depletion of sulfhydryl groups and interference with certain enzymes in the beta-cell (Rerup, 1970). However, the action of alloxan appears to be mediated by producing damage to the beta cells by producing damage to the cell membrane as suggested by permeability studies (Watkins *et al.*, 1964, 1973), rather than through a primary intracellular action. Alloxan has toxic effect on beta-cells of islets of Langerhans through the lipid peroxidation. The process of lipid peroxidation is normally initiated by direct attack of a foreign free radical on unsaturated lipids or by the reaction of singlet oxygen or hydroxyl radicals generated by foreign compounds. As a result of chain reactions in presence of oxygen formation of lipid alcohols, lipid aldehydes and oxydative destruction of cellular lipids occur. Some of these lipoidal derivatives have direct toxic effect on membranes of endoplasmic reticulum and/or lysosomes. Thus toxic effect of alloxan on secretory activity of beta cells is through damage of endoplasmic reticulum.

Functions of secretory cells of various glands including these salivary glands depend on synchronized phenomena such as :

(1) Uptake of water, electrolytes, metabolites, vitamins, etc. from the blood.

(2) Intracellular metabolic activities and

(3) Release or secretion of product(s).

Most of the secretory functions of salivary glands are regulated by autonomous nerves and certain hormones; generally in synchronized way or synergistically. Bloom *et al.*,(1981)and Emmelin(1981), have reported that function of salivary glands is controlled by nerves. Mechanism by which hormones and neurotransmitters regulate secretion of exocrine glands apparently involves the regulation of transmembrane movements of electrolytes, a process for which either cAMP or $Ca^{++}$ serve as second messengers. However, the information about the functions of salivary glands is minimum in endocrine disorder like diabetes. Thus the main objective of present investigation is to understand :

(1) Alterations in secretory functions of submaxillary and parotid salivary glands in normal and diabetic condition in male albino rats.

(2) Role of insulin in the control of

(a) Uptake of required material by these salivary glands from blood.

(b) certain metabolic activities and

(c) secretory activity of cells in alveolar region and reabsorption by tubular region of these glands.

(3) Possibility of diagnostic application of saliva in monitoring diabetic condition.

All the aforesaid studies may demonstrate the involvement of insulin in the metabolic activities in salivary glands and composition of saliva. Most of the work that have been reported deal with mild insulin deficiency induced by single dose of beta toxicant, alloxan or streptozotocin. Although, several studies in this field have reported on varied influences of neuroactive agents on the salivary glands, information regarding pathophysiology, effects on various metabolic aspects due to acute deficiency of insulin are almost lacking. Hence, to have an insight of the acute effects of administration of beta toxicant drug the alloxan, a study was carried out to investigate alterations induced in the metabolic pattern of submandibular and parotid salivary glands after administration of alloxan which is a cytotoxic agent that experimentally induce chronic insulin deficient state in the animals such as male albino rats, most of the rodents and other mammals used in laboratories. Study of various parameters were carried out after ten days of alloxan administration.

The total weight of animal, weight and relative weight of salivary glands, metabolites like glycogen, total lipid, total cholesterol, ascorbic acid content, enzyme activities of $\alpha$-amylase, total adinosine triphosphatase (ATPase), succinic dehydrogenase (SDH), nonspecific acid and alkaline phosphatases and nucleic acid (DNA and RNA) contents, were assayed after ten days of alloxan administration. To ensure the acute diabetic condition the level of blood sugar was measured quantitatively after the five doses of alloxan administration. The results are dealt within the following chapters.

Our study is of interest in view of the fact that physiology and biochemistry of submandibular and parotid salivary glands were stimulated owing to its involvement in various diseases. Enlargement of salivary glands is found in large number of diseases (Rauch, 1959; Thoma and Goldman, 1960; Seifert, 1964 and 1966). In diagnosis special consideration is given to sialdentis, the salivary glands tumors and disturbances of secretion (Dyschylia), it occurs mainly due to sialadenosis and sialothiasis; sialadenosis is a frequent non-inflammatory condition of salivary glands. Occuring usually due to disturbances of glandular metabolism as well as secretion of saliva. The condition is normally not associated with pain and involves bilateral enlargement of the glands especially the parotid (Seifert, 1964 a,b,c and 1966). Sialadenosis is frequently concomitant with pluriglandular disorders such as diabetes mellitus, hypofunction of gonads, Kwashiorkor syndrome, Cirrhosis of liver and chronic alcoholism, hyperinsulinemia has been reported in many diabetic patients treated by modern intensified insulin regimns, its magnitude, frequency,its significance (as an atherogenic factor for example) must be further investigated. Monitoring of diabetic condition requires collection of blood sample at regular intervals. It is actually a painful job to prick lancets every time and it is also equally troublesome to carry out assay of blood glucose. Therefore it is our hope, that if we can find out regular changes in certain definite parameters regarding composition of saliva then this information can be helpful in knowing in a non invasive manner about the stage of diabetic condition and also in monitoring glycemic condition for accurate treatment. Diabetic patients will not require to bear pricking pain every time. Some time recurrent pricking also leads to wound formation which is very difficult to cure.