CHAPTER VI

A QUANTITATIVE STUDY OF ACTIVITIES OF NON-SPECIFIC ACID AND ALKALINE PHOSPHATASES IN THE SUBMAXILLARY AND PAROTID SALIVARY GLANDS OF NORMAL AND ALLOXAN TREATED DIABETIC MALE ALBINO RATS.

Nonspecific acid and alkaline phosphatases are a group of enzymes, which not only hydrolyses, phosphate esters but also involved in transport of metabolites across cell membrane. Omnipresent distribution of one or the other phosphatase in different tissues is suggestive of their role in several cellular activities such as, absorption, secretion, cellular phagocytosis, protein synthesis and many phosphorylating reactions. Metabolic disorders due to insulin deficiency are manifested by affecting the enzyme activities in various cells. Most of the phosphatase group of enzymes are affected in diabetes.

Acid phosphatase being a hydrolitic enzyme is reported to be involved in number of activities such as phagocytosis (Klockars and Wegelius, 1969), dissolution of tissue components (Weber and Niehus, 1961), synthetic activities (Sauter, 1967), protein synthesis (Singer, 1964; Kokko, 1965 and Pears, 1968) differentiation (Ghirette, 1950), repair of skin of many vertebrates (Reiner *et al.*, 1957; Moretti and Mescon, 1956) in primates (Carranza and Cabrini, 1962), in rats (Raekallio, 1960), in guinea pigs (Noback and Patt, 1951), wound healing and repair in diabetic rat liver (Kishnani, 1976), wound healing and repair in diabetic rat skin (Kathuria, 1976). Acid phosphatase was reported in lymphocytes (Elves, 1966). This enzyme is associated with lysosomes (Duve *et al.*, 1962 and Novikoff, 1963) and helps in intracellular digestion of phagocytosed exogenous material. Acid phosphatase has been reported to be localized in the golgi zone of epithelial cells of the intestinal villi of rat and is associated with mucin formation (Ogata *et al.*, 1964). Activity of acid phosphatase was comparatively higher in hepatic cells in graminivorous birds than that of omnivorous or insectivorous. The high histochemical reactivity of acid phosphatase activity in hepatic sinusoids (Shah *et al.*, 1972), and intestinal mucosa indicates its involvement in uptake glucose from blood and glycogenesis in the cells.

Insulin deficiency is found to affect the activities of these two non specific phosphatases (Sneer *et al.*, 1970; Shevchuk 1973), with wide spectrum of substrates and involvement in many activities they reported a significant increase in the activity of acid phosphatase in the serum and decrease in the liver of alloxan treated rats. It is interesting to note that hepatic cells show decreased synthesis of glycogen in diabetic condition. Bagdate *et al.*, (1972), while comparing the phagocytic functions of diabetic rat with that of non-diabetic ones have shown that there was a marked reduction in the phagocytosis in diabetic rats.

Parasympathetic stimulation is also known to increase acid phosphatase activity of submandibular glands (Garrett and kidd, 1977).

The alkaline phosphatase is reported to associated with carbohydrate metabolism (Rosenthal *et al.*, 1960), formation of fibrous protein (Moog, 1946; Bradfield, 1950), calcification of bones (Moog, 1944; Pritchard, 1952), phosphate transfer in DNA metabolism (Rogers, 1960) transport of metabolites across the cell membrane (Bradfield, 1950; Danielli, 1954) and in the formation of mucopolysaccharides of the ground substance (Searcy, 1969) and collagen formation (Fell and Daniell, 1943). Out of its several functions alkaline phosphatase plays an important role in the absorption of fat, carbohydrates and amino acids. A correlation between alkaline phosphatase and the synthesis of phospholipids indicates its involvement in this function (Koyama and Ono, 1960). Short chain fatty acids induce alkaline phosphatase activity in cultured mammalian cells. Thus, they have reported that there exists a relationship between short chain fatty acids and alkaline phosphatase activity in the cellular environment. Alkaline phosphatase is known to be involved in absorption of lipids (Rufo *et al.*, 1973) and lipids as well as amino acids (Pilo *et al.*, 1977) have also reported involvement of liver type of isoenzyme of alkaline phosphatase in absorption of fat in the intestine of nestlings of pigeons.

Moreover, a role of alkaline phosphatase in protein synthesis has also been suggested by Moog (1946). It seems possible that both the phosphatases in one respect or other are involved in protein synthesis but the activity of one or the other in a particular cell depends upon cellular environment, especially the pH.

A higher incidence of elevated circulating activity of alkaline phosphatase has been reported in human and the experimental animal in diabetes mellitus (Camerini *et al.*, 1962; Belfiore *et al.*, 1978; Stepan *et al.*, 1980), also an increased activity of the bone isoenzyme of alkaline phosphatase in serum has been found in diabetes and was shown to have a significant positive correlation with urinary hydroxyproline excretion similar to osteoporosis (Stepan *et al.*, 1980), loss of bone mass (Levin *et al.*, 1976) and mineral content (McNair *et al.*, 1979) in diabetes mellitus. With experimental diabetes in the rat an elevated serum alkaline phosphatase activity following alloxan injection has been known for some time (Cantor *et al.*, 1947). Marked increase of alkaline phosphatase activity in serum of diabetic B.B. Wistar rats was shown by Scott *et al.*, (1983). The elevated circulatory activity of alkaline phosphatase in alloxan or streptozotocin diabetes has been attributed to an isoenzyme of intestinal origin (Chua and Shrago, 1978; Hough *et al.*, 1981). Alkaline phosphatase activity in homogenates of liver and bone during chronic streptozotocin induced insulin deficiency in the rat was found to be unchanged or decreased respectively (Hough *et al.*, 1981).

This is not surprising as diabetic complications may directly or indirectly involve liver, bone, intestine and tissue or glands, which contribute enzyme molecules into blood plasma. Insulin affects the functions of many parts of alimentary and accessory digestive glands. Salivary glands are also important accessory digestive glands. However, alteration in the activities of these two phosphatases in salivary glands in diabetic condition still remains ill defined. This study was therefore undertaken to observe the nature of the alterations in activities of acid and alkaline phosphatases of submaxillary and parotid salivary glands of normal and alloxan treated diabetic male albino rats.

Materials and Methods

Healthy male albino rats weighing 120 $\pm$ 30 gms were used as experimental animals. The rats were maintained on a balanced diet and were provided with water *ad libitum*. The saline and alloxan treatment to the control and experimental groups of rats were given as mentioned earlier in the chapter-I. The rats were sacrificed after the treatment of respective duration by cervical dislocation under mild ether

anaesthesia. The submaxillary and parotid glands were excised and were made free of connective tissue and were weighed on the Mettler balance. The ice-chilled homogenate was prepared using chilled mortars and pestles in chilled distilled water. The quantitative estimation of acid and alkaline phosphatases was carried out by employing the method of Bessey and Lowry (1946) (Sigma technical bulletin No. 104) using p-nitrophenyl phosphate as substrate. The values obtained are expressed in terms of μ-mole-p-nitrophenol released/mg protein/30 minutes. Protein content of homogenate was estimated by employing the method described by Lowry *et al.*, (1951). The statistical analysis was done using Student's 't' test.

Results :

Activity of acid phosphatase of submaxillary gland is slightly higher i.e., 0.016 than that of parotid which is 0.013 μ-mole p-nitrophenol released/mg protein/30 minutes in the normal rats. Submaxillary gland did not register any alteration but parotid gland showed smaller but significant ($P < 0.005$) increased value in diabetic rats.

Activity of alkaline phosphatase of submaxillary gland is much higher than that of parotid in normal rats. It is 0.011 in submaxillary and 0.004 in parotid in normal condition, submaxillary gland showed significant ($P < 0.05$) decrease whereas activity of alkaline phosphatase of parotid in diabetic rats failed to show alterations like acid phosphatase in submaxillary gland (Table VIa and Fig VIb).

Discussion

A comparatively higher acid phosphatase activity of submaxillary gland than that of parotid gland of normal rats indicate its involvement in uptake of glucose from blood and its increased utilization in synthesis of mucin. Being a mixed type of salivary gland, mucous acini are known for their activity of synthesis and secretion of mucin a mixture of complex mucopolysaccharides, an important component of saliva, known for its smoothening action, Gangaramani (1976) had observed higher acid phosphatase activity in liver of albino rats and also co-related it with uptake of

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Level of blood glucose, activities of nonspecific acid and alkaline phosphatases in the submaxillary and parotid salivary glands of normal, control and diabetic male albino rats. Mean ± SD.

Physiological condition of rat	Blood glucose level[1]	Acid phosphatase[2]		Alkaline phosphatase[3]	
		submax. gland	parotid gland	submax. gland	parotid gland
Normal	127.65 ± 9.25	0.016 ± 0.001	0.013 ± 0.001	0.011 ± 0.002	0.004 ± 0.001
Control	123.25 ± 11.53	0.017 ± 0.001	0.015 ± 0.001	0.009 ± 0.001	0.004 ± 0.001
Diabetic	257.04 ± 15.32	0.016 ± 0.001	0.017 ± 0.001	0.008 ± 0.001	0.004 ± 0.001
Significant (*p) at the level	P<0.001	NS	P<0.005	P<0.05	NS

1. mg blood glucose/100 ml. blood (as an index of diabetic state)
2. μ-mole p-nitrophenol released/mg protein/30 min.
3. μ-mole p-nitrophenol released/mg protein/30 min.

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

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

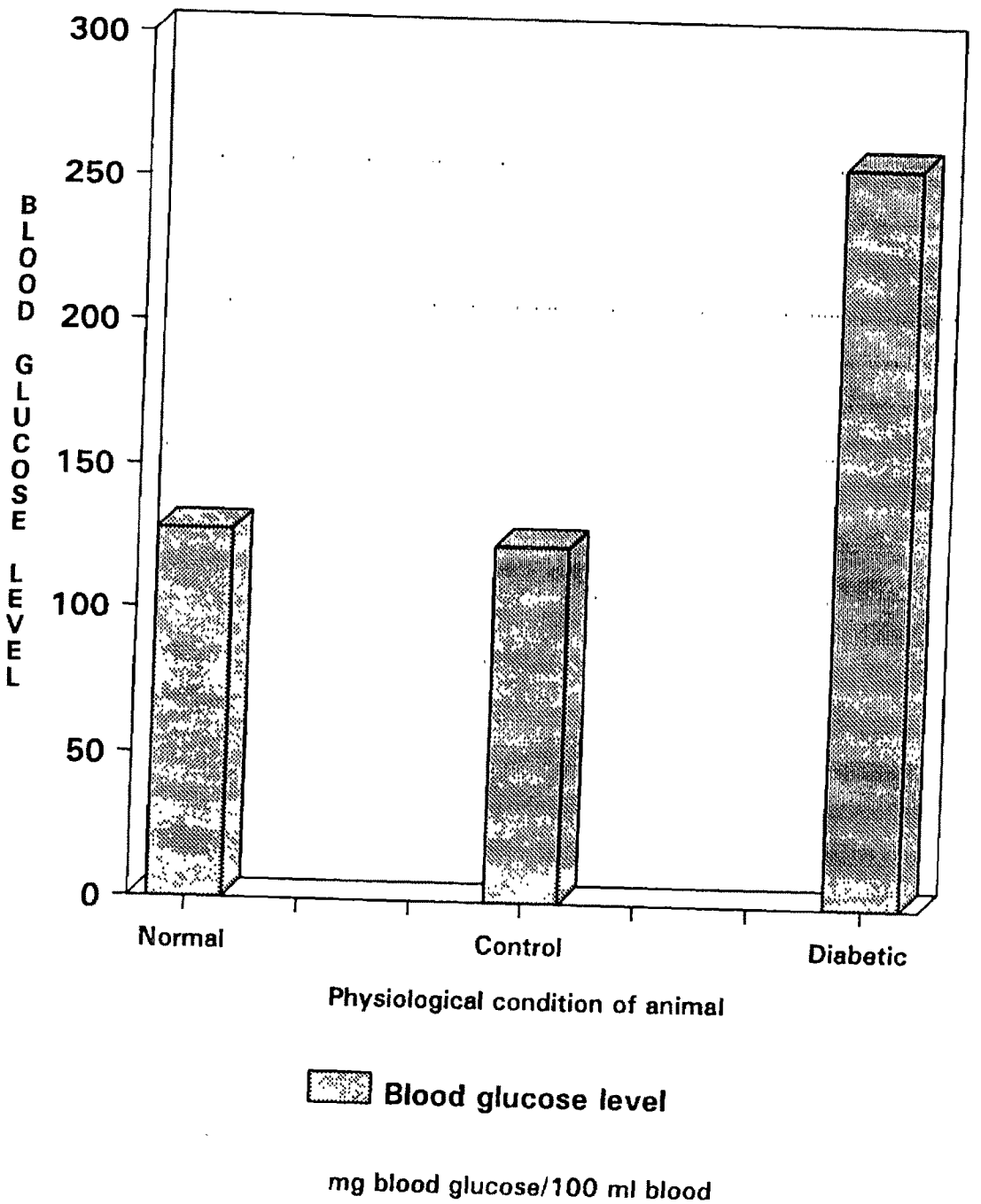

Fig.VIa. Graphic presentation of Blood Glucose Level of normal, control and diabetic male albino rats.

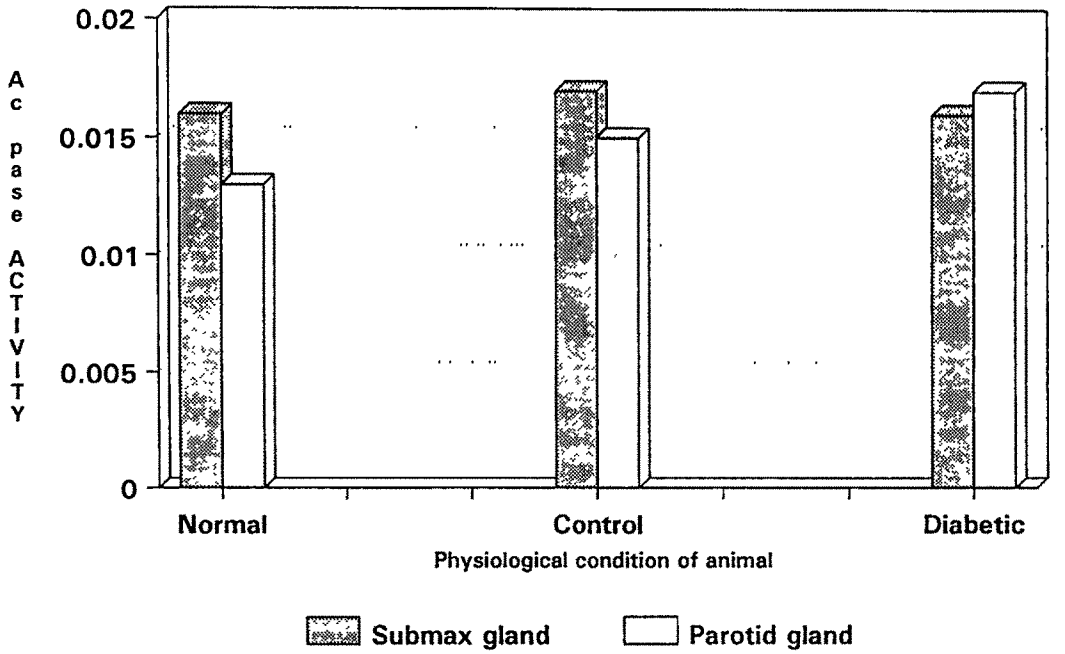

Fig.VIb. Graphic presentation of Acid Phosphatase Activity of submaxillary and parotid salivary glands of normal, control and diabetic male albino rats.

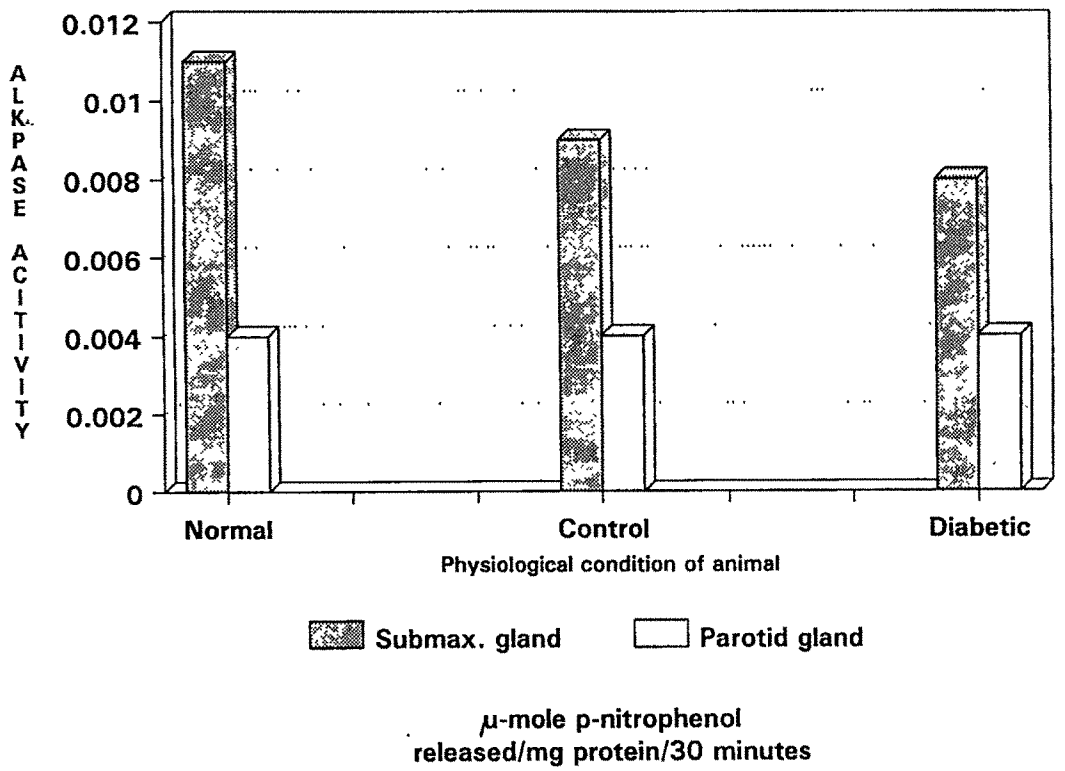

Fig.VIb. Graphic presentation of Alkaline Phosphatase Activity of submaxillary and parotid salivary glands of normal, control and diabetic male albino rats.

glucose and its metabolism, Pilo *et al.*, (1977), have also noticed progressive increase in activity of non-specific acid phosphatase from the lowest value of one day upto the 20th day in the liver during post hatching development of nestling of pigeon. It was suggested that peak level of acid phosphatase on 20th day after hatching coincided with the increasing glucose load from intestine as at this stage nestlings are given carbohydrate rich grains and crop milk is discontinued. However, it is interesting to observe that higher values of glycogen and lipid of submaxillary gland of diabetic rats failed to show any noticeable increase in activity of acid phosphatase. This support the concentration that (1) uptake of glucose by the glands is not affected in diabetic condition (2) salivary glands might be having type-I hexokinase which is insulin independent isoenzyme and (3) increased content of glycogen and lipids is not due to higher glycogenesis and lipogenesis but due to accumulation reflected due to subnormal synthesis of secretory components such as mucopolysaccharides. Parotid gland of diabetic rats showed increased activity of acid phosphatase which is significant at the level $P < 0.005$. Certain phosphatases are known to be active at an acidic pH and have certain supplementary functions in secondary pathway of carbohydrate metabolism. Liver cells release glucose by hydrolysing glucose-6-phosphate with the help of enzyme-glucose-6-phosphatase, which require medium with acidic pH. In parotid glands of diabetic rats high acid phosphatase activity could be due to activation of glucose-6-phosphatase to a certain extent. In certain conditions related to change in endocrine conditions such as gestation period saliva do contain glucose as an abnormal constituent and pregnant women feel sweetening in mouth and show temptation or preference for having edible items with contrasting tastes for a change such as salty, sour etc. Sweet saliva and susceptible mucosal lining in mouth provide ideal medium for easy growth of oral pathogen and probably this is the reason for higher frequency of infections in mouth of diabetic patients. Similarly, in diabetic condition one can predicate release of glucose from the acinar cells of this salivary glands. Inhibition of protein synthesis or increased protein degradation is normally expected in salivary glands of diabetic rats in deficiency of insulin. Because in several tissues insulin inhibits protein degradation. This protein degradation has been observed in muscles (Jefferson *et al.*, 1977) and perfused rat liver (Kanter, 1976; Mortimore and Mondon, 1979). A considerable activity of the protein degradation of cells takes place in lysosomes. Reduced protein synthesis has been observed indirectly in parotid glands of diabetic rats which have displayed very low α-amylase activity (chapter I). Thus, increased activity of non-specific acid phosphatase could be actually due to total activities

of all these phosphatases described above.

The activity of alkaline phosphatase showed much similar but distinct difference between two types of salivary glands in normal rats. It was much higher in submaxillary gland in normal condition. These two types of salivary glands showed quite a different pattern. Activity of alkaline phosphatase showed significant decrease ($P < 0.05$) in submaxillary gland and remained unaltered in parotid gland. In case of acid phosphatase it was unaltered in submaxillary but increased in parotid.

Non specific acid and alkaline phosphatases are a group of enzymes which not only hydrolyses phosphate esters but also helps in transport of metabolites and secretory material across plasma membrane. Wide spread distribution of one or other phosphatase in different tissues is suggestive of their role in several cellular activities eg. absorption, secretion, and cellular phagocytosis. A progressive increase in the activity of acid phosphatase and gradual decrease of alkaline phosphatase activity is found to be related to glycogen accumulating capacity of embryonic liver of chick (Moog, 1965). However, alterations in activities of these nonspecific phosphatase in liver would not show similar or parallel changes in other glands such as salivary glands. In liver, accumulation of glycogen depends on several phenomena such as uptake of glucose, metabolism of glucose inside cell, synthesis of glucose and glycogen from non-carbohydrate molecules and hydrolysis of glycogen and release of glucose from hepatic cells. Whereas in normal condition level of glycogen in the acinar cells of salivary glands depends on uptake of glucose from blood and its metabolism in the cells. Shah *et al.*, (1972) have observed comparatively higher histochemical reactivity of alkaline phosphatase in hepatic sinusoidal lining of insectivorous and omnivorous birds than that of graminivorous birds. Pilo *et al.*, (1977) have observed the highest activity of alkaline phosphatase in liver of one day old nesting of pigeon. These authors also observed gradual decline in its activity. The high alkaline phosphatase activity in liver of insectivorous and omnivorous birds as well as in the liver of one day old pigeon indicate the involvement of alkaline phosphatase in uptake of aminoacids, fatty acids and glycerol. Very strong histochemical localization of alkaline phosphatase on the brush border of proximal convoluted tubule in the kidney also support involvement of this enzyme in uptake or transport or absorption of metabolites. However, from the information available at present, it could be noticed that except just a histochemical localization, no one has

tried to study these two phosphatase in two different types of salivary glands in normal as well as in diabetic condition of experimental animals.

Reduction in activity of alkaline phosphatase coincided with the accumulation of glycogen content in submaxillary glands in diabetic rats, such correlationship has been suggested by Moog (1965), in liver of chick. It is possible that alkaline phosphatase involved in secretory action of submaxillary gland was affected. Activity of ∝-amylase showed significant decline in salivary gland of diabetic rats (Chapter I). Similarly such parallel reduction in synthesis and secretion of mucopolysaccharides or mucin from the mucous acini of gland could be predicted. It certainly require further investigation. On the other hand parotid glands of diabetic rats failed to show any kind of alteration in its alkaline phosphatase activity from its value of normal rats. This fact indicated that mechanism of uptake of metabolites from blood to the cells of serous types of acini is not affected much. Accumulation of metabolities, lipids etc in parotid gland of diabetic rats suggest that metabolism of serous acini had affected. Reduced ∝-amylase activity in parotid glands of diabetic rats do indicate that synthesis and secretion of this vital protein molecule is reduced. However, apocrine nature of secretion of this ∝-amylase containing watery secretion does not involve membrane bound enzyme like alkaline phosphatase. Therefore, activity of alkaline phosphatase of parotid salivary gland did not register any alteration.

It could be concluded from the unaltered activity in submaxillary and elevated activity of acid phosphatase in parotid gland of diabetic rats, that probably glucose uptake is not much affected or reduced in these glands even in deficiency of insulin. Signigicantly elevated level of acid phosphatase in parotid gland of diabetic rats could be due to activated glucose-6-phosphatase and/or lysosomal enzymes. Under this situation only one can expect exit of glucose along with secretion of serous acini. Comparatively, higher activity of alkaline phosphatase in submaxillary glands of normal rats could be due to its active roles in secretory activity of acini specifically mucous type/secreting complex mucin and tubular reabsorption also. Significant decline in activity of alkaline phosphatase of submaxillary gland of diabetic rats also support above mentioned possibility. Unaltered activity of alkaline phosphatase in parotid gland of diabetic rats suggest that it is not much involved in function of this gland.