# CHAPTER V

## A QUANTITATIVE STUDY OF ASCORBIC ACID AND CHOLESTEROL IN SUBMAXILLARY AND PAROTID SALIVARY GLANDS OF NORMAL AND ALLOXAN TREATED DIABETIC MALE ALBINO RATS.

Ascorbic acid appear to act as a cofactor in enzymatic hydroxylation of proline residue of the collagen of connective tissue of vertebrates to form 4-hydroxyproline. Thus ascorbic acid play very significant role in formation and maintenance of connective tissues. It is vitamin only for human beings and a few other vertebrates. Most of the animals and probably all plants synthesize L-ascorbic acid from glucose. Liver is the main site of ascorbic acid (AA) synthesis and generally other organs or tissues depend on the supply of AA from liver through blood. AA is believed to play an important role in metabolic reactions as an $H^+$ acceptor, there by preventing many enzymes and cofactors from being rapidly reduced during oxidation-reduction reactions. Apart from assisting in haematopoiesis, immunity, resistance to cold stress, AA is involved as Roe(1954), has suggested, in the formation of intercellular materials i.e. in synthesis and maturation of reticulin, collagen, matrix of skeletons, dentine and metabolism of mucopolysaccharides.

Ascorbic acid is also known to be associated with metabolism of carbohydrates (Banerjee and Ghosh, 1947; Banerjee and Ganguli, 1962), lipid (Rush and Kline, 1941) and protein, especially for maintaining tyrosin oxidation (Levine *et al.*, 1941).

From the previous study carried out in this laboratory on AA, concentration in liver in some condition such as diabetes (Kishnani, 1976) and castration (Ambadkar and Gangaramani, 1980), it was concluded that hormones like insulin and testosterone affect not only several metabolic activities but also synthesis of AA. It has been reported that the skin of diabetic rats contains subnormal quantity of connective tissue components such as mucopolysaccharides, glycoproteins and collagen (Berenson *et al.*, 1972). Consequently, a delayed wound healing is also observed in the skin of diabetic rats (Kathuria, 1976), this is due to the subnormal level of collagen in the skin. In the light of the literature cited above on varied and multiple roles of ascorbic acid in many organs in several physiological and endocrine conditions, it was thought desirable to study the levels of AA in two different types of salivary glands in normal as well as in diabetic condition in male albino rats.

High levels of plasma cholesterol have been frequently found in human diabetes and also develop in alloxan diabetic rat (Wong and Bruggen, 1960). Administration of alloxan in rats results in many metabolic abnormalities, including hyperlipidemia, cholesterol (Bernard *et al.*, 1979). Hepatic cholesterol synthesis has been reported to be accelerated (Hotta and Chaikoff, 1952), normal (Foster *et al.* 1960; or even depressed (Wong *et al.*, 1960; Claremburg *et al.*, 1966). It has been postulated (Wong *et al.*, 1960) that the synthesis of bile salts could be depressed in these diabetic animals.

It has been shown that HMG-CoA reductase is the key enzyme in cholesterol biosynthesis (Horton *et al.*, 1970; Bortz and Stelle, 1971; Bortz *et al.*, 1973; Gregory and Booth, 1975). Higher rate of De Novo synthesis of cholesterol in the intestinal wall of diabetic rat is also reported by Nervi *et al.*, (1974). Higher level of cholesterol in the liver has been observed in castrated rats (Ambadkar and Gangaramani, 1976) and in alloxan treated diabetic rats (Kishnani *et al.*, 1977). Actually, salivary glands are not much known for their involvement in metabolism of cholesterol like liver. However from their study, it has become clear that gonadal hormones do have influence on metabolism of cholesterol in salivary glands. Booth (1972), has reported about occurrence of testosterone and 5-$\alpha$-dihydroxyacetone in the submaxillary salivary glands of boar. Katkov *et al.*, (1972) and Booth (1977) have studied metabolism of androgens in submaxillary glands of boar in their *in vitro* experiments. Ferguson *et al.* (1970) have demonstrated histochemical localization of activities of certain steroid dehydrogenases. These reports indicate that the mammalian salivary glands do have a capacity to metabolize steroid hormones, which is also influenced by hormones. Very recently Desai (1989), has reported increased cholesterol level in submaxillary salivary glands of castrated rats. To understand the influence of insulin on cholesterol metabolism of submaxillary and parotid salivary glands a quantitative evaluation of cholesterol has been carried out in these glands of normal and alloxan treated diabetic male rats.

## Materials and Methods

Healthy male albino rats weighing 120 gms to 150 gms. were used. The rats were acclimatized to laboratory conditions for few days and were maintained on balanced diet and were provided with water *ad. libitum*. The procedures for treatments of saline in control and alloxan for inducing diabetes in rats of experimental groups were same as described in the chapter I. The rats were sacrificed by cervical dislocation under mild ether anaesthesia. A sample of blood was collected from external jugular vein for estimation of blood glucose level by the method of Folin and Malmros (1929). Submandibular and parotid glands of both the sides were quickly excised, freed of connective tissue, and weighed accurately on a Mettler balance. Glands of one side of submandibular and parotid were utilized for the assay of total ascorbic acid content. For estimation of ascorbic acid content the glands were homogenized in chilled 6% tricholoracetic acid (TCA), in chilled mortars. The total ascorbic acid was estimated using the aliquots of these extracts by following the method of Roe and Kuether (1943) as described by Roe (1954), using dinitrophenyl hydrazine. The total ascorbic acid was calculated in terms by percentage of the fresh tissue weight of submandibular and parotid glands i.e. mg ascorbate/100 mg fresh gland. The glands from other side were used for determination of total cholesterol content. For the estimation of cholesterol the weighed tissue were homogenized in acetic acid separately. The aliquots of these glands were used for assay of total cholesterol content by following the method of Crawford (1958) using ferric chloride a colour reagent. The total cholesterol content of these glands were calculated in terms by percentage of fresh tissue weight of submandibular and parotid glands i.e. mg cholesterol/100 mg fresh gland. Data were analyzed using Student's 't' test.

## Results :

The data on the levels of blood glucose ascorbic acid and cholesterol of submaxillary and parotid of normal, control and alloxan treated diabetic rats are presented in table V and fig Va and Fig Vb. Data of blood glucose is taken over here as an index of diabetic state. It is 127.75 mg blood/100 ml of blood in normal rats, but significantly higher i.e. 257.04 mg blood glucose/100 ml of blood in diabetic rats.

Table V

Level of blood glucose, ascorbic acid content and total cholesterol content in submaxillary and parotid salivary glands of normal, control and diabetic male albino rats. Mean ± SD.

| Physiological condition of animal | Blood glucose level[1] | Ascorbic acid content[2] | | Cholesterol content[3] | |
|---|---|---|---|---|---|
| | | Submax. gland | parotid gland | Submax. gland | parotid gland |
| Normal | 127.75 ± 8.95 | 0.030 ± 0.001 | 0.033 ± 0.001 | 0.221 ± 0.002 | 0.237 ± 0.012 |
| Control | 123.25 ± 8.96 | 0.030 ± 0.001 | 0.035 ± 0.001 | 0.221 ± 0.012 | 0.239 ± 0.009 |
| Diabetic | 257.04 ± 15.20 | 0.037 ± 0.001 | 0.036 ± 0.001 | 0.270 ± 0.007 | 0.289 ± 0.011 |
| Significant (*P) at the level | P<0.001 | P<0.001 | NS | P<0.01 | P<0.001 |

1. mg blood glucose/100 ml. blood (As and index of diabetic state)
2. mg ascorbic acid/100 mg of fresh gland.
3. mg cholesterol/100 mg of fresh gland

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

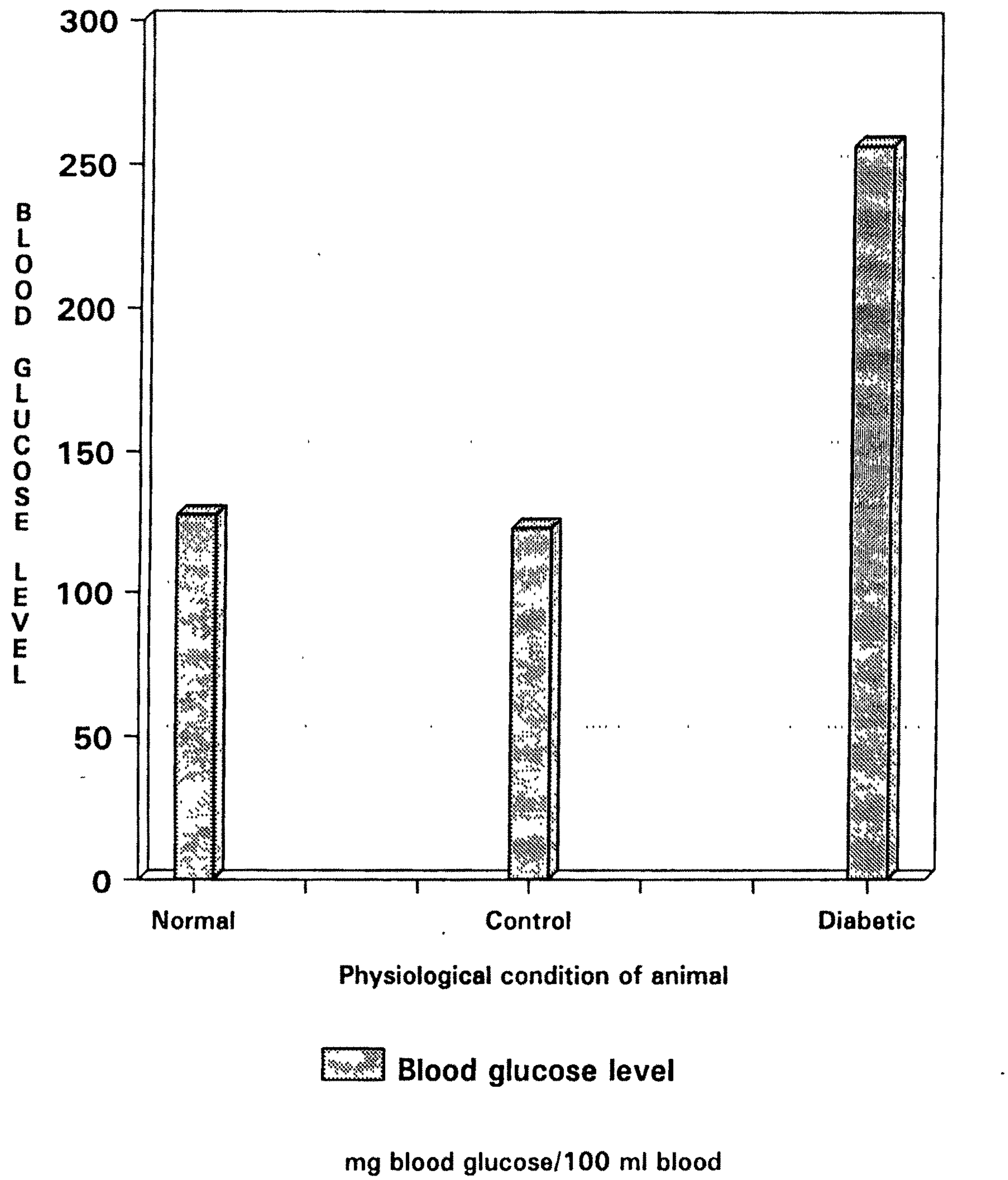
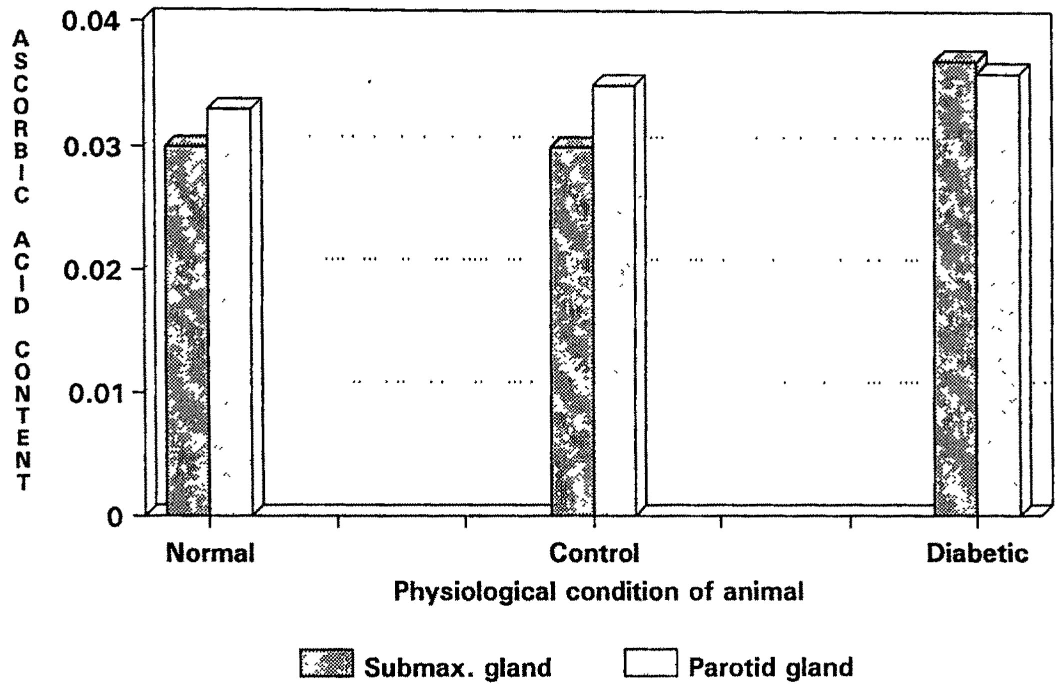
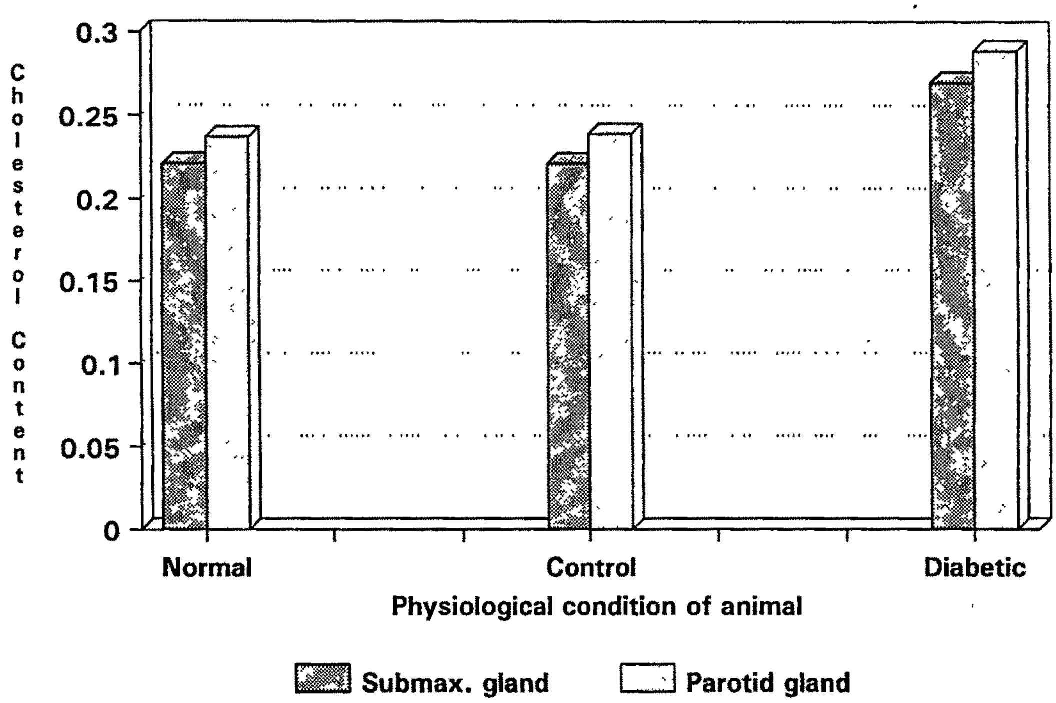



Fig.Va. Graphic presentation of Blood Glucose Level of normal, control and diabetic male albino rats.




mg ascorbic acid/100 mg fresh gland

Fig.Vb. Graphic presentation of Ascorbic Acid content of submaxillary and parotid salivary glands of normal, control and diabetic male albino rats.




mg cholesterol/100 mg fresh gland

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

Levels of ascorbic acid of both the types of salivary glands are more or less similar i.e. 0.030 mg ascorbic acid/100 mg of fresh gland in submaxillary gland and 0.033 mg ascorbic acid/100 mg of fresh gland in parotid gland of normal animals. Submaxillary gland of diabetic rat showed significantly higher value of i.e. 0.037 mg ascorbic acid/100ml of fresh gland ($P < 0.001$) but parotid gland of diabetic rat failed to show significantly higher value i.e. 0.036 mg ascorbic acid/100 mg of fresh gland. In terms of percentage of normal value, submaxillary gland showed 23.3% increment and parotid gland showed only 9.1%.

In normal rats cholesterol content of submaxillary gland is 0.221 mg cholesterol/ 100 mg fresh weight, where as that of parotid gland is 0.237 mg cholesterol/100 mg fresh gland weight. Thus, parotid gland showed little higher cholesterol content. Both the types of salivary glands showed significant increased value of cholesterol in diabetic rats. The cholesterol content of submaxillary gland was 0.270 mg/100 mg fresh weight which is significant at the level of $P < 0.01$ and was 22.17% of the normal value. Parotid gland showed 0.289/mg cholesterol/100 mg fresh weight, which is significant at the level of $P < 0.001$ and was 21.94% of the normal value.

## Discussion

Since ascorbic acid is synthesized in liver of albino rats, its level in this organ varies according to the rate of synthesis and/or release of AA in to blood for distribution to all other organs of body for their functions, or even its utilization in the liver itself. Like all other glands, salivary glands also receive vitamin-C or ascorbic acid from liver through blood. Thus level of ascorbic acid in the salivary glands depends on many factors such as uptake or supply of ascorbic acid from blood to salivary glands, its utilization for the function in the gland and its normal or abnormal metabolism in the glands.

From the data of ascorbic acid presented in Table V and figure V, it is clear that both mixed type (submaxillary) as well as serous type (parotid) of salivary glands showed considerably equal amount of ascorbic acid. Both the types of salivary glands showed increased or higher value of ascorbic acid in diabetic condition. The increase is significant in submaxillary but is not significant in parotid gland in diabetic rats.

Considering the association of ascorbic acid with carbohydrate metabolism (Banerjee and Ghosh, 1947; Banerjee and Ganguli, 1962) lipid metabolism (Rush and Kline, 1941) and protein metabolism (Levine *et al.*, 1941), it is assumed that its occurrence in both the types of salivary gland is essential for normal metabolic activities like other vital and active glands.

Saliva is slightly viscous, colourless and opalescent secretion which is actually mixture of many salivary glands opening into mouth. It contains, water, inorganic ions, organic components like glycoproteins, mucin, enzyme, α-amylase (ptyalin), aminoacids, lipids, trace of urea, citrates. Glycoproteins of saliva include neutral as well as sulfated mucins. Blood group substances are also one of the components of salivary glycoproteins. Mucin is rich in several glycoproteins and mucopolysaccharides. Therefore one can expect existence and active role of ascorbic acid in mucous acini of submaxillary salivary glands. Berenson *et al.*, (1972) have suggested that subnormal level of connective tissue components such as mucopolysaccharides, glycoproteins and collagen etc. is due to deficiency of insulin in skin of diabetic animals. The metabolism of L-ascorbic acid is known to be altered under various physiologically abnormal conditions including that of adrenalectomy, hypophysectomy and alloxan diabetes (Majumder *et al.*, 1973). Delayed wound healing is observed in liver of alloxan treated diabetic rats (Kishnani *et al.*, 1977) is also due to subnormal level of connective tissue components. Similarly insulin deficiency or insufficiency affect the metabolic activities and thus secretory activities of salivary glands. Higher values of certain metabolites due to accumulation resulted from reduced metabolism or use has indicated subnormal function of both the types of glands in diabetic rats (Chapter II). Even decreased activities of SDH and ATPase in both the types of salivary glands of diabetic rats (Chapter V) have indicated slow operation of Krebs cycle and oxidative phosphorylation.

Hence higher level of ascorbic acid in salivary glands of diabetic rats may be due to its increased uptake from blood as well as due to accumulation resulted from its reduced utilisation. It is very interesting to notice that submaxillary gland has shown higher percent of accumulation i.e. 23.3%, which indicates that synthesis of mucin with its several glycoprotein and mucopolysaccharide components, is reduced and as a result submaxillary gland of diabetic rats showed much higher value, whereas parotid gland showed only non significant and small increase i.e. 9.1%. As parotid gland secretes watery secretion rich in $\alpha$-amylase, it is made of serous acini only. Therefore reduced value of ascorbic acid in parotid gland seems to be due to reduced metabolic activities. As synthesis of glycoprotein and mucopolysaccharides is not taking place in serous acini of parotid glands of diabetic rats, hence the AA content is less in this gland. Wong and Bruggen (1960) have reported hypercholesterolemia in alloxan treated diabetic rats. Hepatic content of cholesterol also increases in diabetic condition. Wong *et al.*, (1960) have opined that this hypercholesterolemia of the diabetic rat could be due to an impairment in the catabolism of cholesterol to bile salts. The observation of a highly significant alterations in the biliary excretion of the bile salts in alloxan induced diabetic rats also support the view of altered cholesterol metabolism. In normal condition synthesis of bile is partly regulated by a negative feed back mechanism that depends on the rate of bile salts returning to the liver through hepatic portal vein (Shefer *et al.*, 1969). Observation of Nervi *et al.*, (1974) proved that the changes in cholesterol level in the plasma and in tissue such as liver are mainly due to alterations in metabolism of cholesterol and not due to direct or indirect toxic effect of alloxan. Watanabe (1971), has observed an increase in compound lipids including cholesterol in liver of alloxan treated diabetic rats. Duncan (1951), found fatty deposits in the liver of untreated diabetic patients and these deposits disappeared quickly, when the disease was controlled.

There are numerous reports correlating castration and gonadal hormonal replacement and levels of total lipids and cholesterol in the accessory reproductive organs and other peripheral organs. Levels of free and estrified cholesterol increases under the influence of antiandrogens, whereas administration of testosterone propionate lowers down the levels. Minetti *et al.*, (1985) have observed that the weight and protein content of submaxillary glands are influenced by administration of testosterone and metabolites. Salivary glands show response to other hormones also. Increased total lipid content of both the types of salivary glands has been observed in alloxan treated male albino rats. The results of present study showed alterations of cholesterol level in both the types of salivary glands of diabetic male albino rats. Both submaxillary and parotid salivary glands showed significant increase in cholesterol level. This evident increase in total cholesterol content of submaxillary as well as parotid salivary glands indicates either reduction in cholesterol catabolism or increased cholesterogenesis. Reduced α-amylase activity observed in serous type cell (chapter I) and reduced activity of SDH and ATPase indicated reduced metabolism in salivary glands and hence surplus or unutilized carbohydrates could be used for cholesterologenesis. The results of present investigation have revealed that not only plasma and liver show high levels of cholesterol but also salivary glands in diabetic condition. Insulin deficiency affect or depress the synthesis of bile salts in hepatic cells and hence such diabetic cells show high cholesterol level. Like long chain fatty acids, cholesterol is synthesized from Acetyl-CoA. Hotta and Chaikoff, (1952), has observed increased rate of incorporation of 14-c-acetate to cholesterol. Intestinal absorption of cholesterol also contribute for higher cholesterolemia. It is quite possible that this hypercholesterolemia may lead or induce transfer of cholesterol from plasma to the cells. Cells of mucous acini and serous acini in the insulin deficiency have subnormal activity. α-amylase activity of both the types of glands has been declined. Thus higher level of cholesterol in both the types salivary glands of diabetic rats could be due to (1) increased synthesis of cholesterol and (2) reduced utilization or catabolism of cholesterol in endocrine disorder such as diabetes mellitus.

Higher levels of ascorbic acid in both the types of salivary glands of diabetic rats indicate that it is due to accumulation reflected from its reduced utilization in subnormal functioning of glands due to the deficiency of insulin. Comparatively, higher percentage of increment in submaxillary gland indicate its greater involvement in synthesis of mucopolysaccharides of mucin. Non significant increment of AA in parotid gland is probably due to its less involvement in function of serous acini. It could be also concluded that deficiency of insulin leads to increased cholesterol level in these salivary glands of diabetic rats. Increased cholesterologenesis or accumulation to reduced utilization could be the reason.