CHAPTER III

A QUANTITATIVE STUDY OF NUCLEIC ACIDS OF SUBMAXILLARY AND PAROTID SALIVARY GLANDS OF NORMAL AND ALLOXAN TREATED DIABETIC MALE ALBINO RATS.

Nucleic acids play a decisive role in many factors such as inheritance of characters, the development and growth of an organism etc. Nucleic acids are associated with increased cell metabolism, growth and protein synthesis (Riddiford, 1960; Hay and Fischman, 1961; Olsteen and Walker, 1961; Wilson 1962; Vollmer and Kauffmann, 1963; Kidson and Kirby, 1964; Liso *et al.*, 1965; Kosto *et al.*, 1967; Minguell and Sierralte, 1975; Kurtz *et al.*, 1976).

Insulin is a major anabolic hormone that regulates the metabolism of most cells. The potency of insulin due to short-term, intermediate and long-term effects (occurring within hours) is the stimulation of RNA and DNA synthesis (Krahl, 1974; Pilkis and Park 1974; Fain 1974; Czech, 1977; Goldfine, 1977, 1978a). In addition to regulating cellular effects that have varying temporal sequence, insulin also regulate functions on a variety of organelles including nuclei, endoplasmic reticulum, lysosomes, mitochondria, cytoplasm and outermost envelop of cell i.e. plasma membrane.

Insulin regulates the synthesis of both DNA and RNA. Insulin has been used for number of decades to stimulate the growth of cell in tissue culture (Gey and Thalhimer, 1924). Usually higher than physiologic concentrations of insulin are necessary for this effect (Smith and Temin, 1974; Rechler *et al.*, 1974) and it is likely that in many instances insulin is interacting with receptors for the various insulin like growth peptides such as somatomedin, MSA or non-suppressible insulin - like activity (NSILA-S), (Smith and Temin, 1974; Rechler *et al.*, 1974; Chochinov and Daughaday, 1976). Inhibition of RNA synthesis by actinomycin has been reported to block the insulin stimulated synthesis of several enzymes in diabetic rats, including fatty acid synthetase, glycogen synthetase, hexokinase, phosphofructose kinase and

pyruvate kinase (Steiner and King, 1964; Steiner, 1966; Weber, 1972; Krahl, 1974). More recent studies have indicated that the production of messenger RNA for albumin is decreased in the liver of diabetic rats and that this diminished level of messenger RNA can be restored by insulin administration in vivo (Peavy *et al.*, 1978). Administration of insulin to diabetic rats both restores diminished pancreatic amylase levels and reduces increased trypsinogen levels while actinomycin treatment blocks this effect (Soling and Unger, 1972). Many reports are available on the effect of neurotransmitters of sympathetic and parasympathetic divisions of autonomic nervous system or even effects of certain gonadal hormones (see Introduction). Very less information is available on the influence of insulin on salivary glands or alterations in salivary glands in diabetic animals. Recently Anderson and Johnson (1981) have reported about both the nucleic acid contents in parotid salivary glands but not on other types of salivary glands. However, the effect of insulin deficiency due to beta-cell toxicant, alloxan or streptozotocin induced diabetes on the content of nucleic acids of different types of salivary glands of rat needs more elaborate understanding. Therefore a comparative study is undertaken with a view to understand nucleic acid content of submaxillary and parotid salivary glands in normal as well as in diabetic conditions.

Materials and Methods

Male albino rats weighing about 120 to 150 gms were acclimatized and maintained in the laboratory on balanced diet and water provided *ad. libitum*. The rats were divided into three groups; normal, control and experimental; and were provided with the respective treatments according to the method described in chapter I, using similar dosages of saline and alloxan. The rats were sacrificed after the prescribed period of treatment by cervical dislocation under mild ether anaesthesia. Parotid and submaxillary glands of both the sides were quickly excised, freed of connective tissue and were accurately weighed on the Mettler balance. Using chilled mortars and pestles, homogenate was prepared in ice-chilled distilled water. Quantitative estimation of nucleic acids content was carried out in this aqueous homogenate employing the method of Schneider (1957). For the estimation of deoxyribonucleic acid (DNA), diphenylamine (DPA) reagent was used and for ribonucleic acid (RNA), orcinol reagent was used. Standard curves were prepared for DNA and RNA separately. Values of DNA and RNA of the homogenate were calculated from the standard

curve, in terms of weight of the fresh gland. Contents of DNA and RNA were expressed as mg DNA/100 mg of fresh gland and mg RNA/100 mg of fresh gland respectively. Statistical analysis of the results were done using Students 't' test.

RESULTS

From the data (Table III and Fig.IIIa and IIIb) it is clear that DNA content of submaxillary gland is 0.431 mg/100 mg, fresh gland which is higher than that of parotid (0.161 mg/100mg fresh weight) in normal condition. RNA content of these two glands showed similar trend in normal condition. It is 0.246 mg/100mg in submaxillary gland and 0.149 mg/100mg in parotid in normal animals.

DNA content of both the glands showed increased values in diabetic animals. It is 0.462 mg/100mg fresh submaxillary and 0.182 mg/100mg parotid gland. These increments in diabetic condition for both the glands are statistically significant at the level of $p < 0.05$ and $p < 0.02$ respectively in submaxillary and parotid glands. Percentage increase of DNA contents of both the glands in the diabetic condition is 7.0 % and 13.0 % in submaxillary and parotid glands respectively.

RNA content of submaxillary and parotid glands of diabetic rats showed non-significant as well as non-parallel changes, it attained the levels of 0.267 mg/100mg fresh gland in submaxillary and 0.133 mg/100mg fresh gland in parotid.

Alterations in terms of percentage of control values are 8.5 % in submaxillary and 10.73 % in parotid gland.

DISCUSSION

From the results presented it is clear that the contents of both the nucleic acids i.e DNA and RNA of submaxillary gland showed higher values than that of parotid gland in normal condition. Both the salivary glands showed increased values of DNA

Table III

Level of blood glucose and nucleic acid content (DNA and RNA) in submaxillary and parotid salivary glands of normal, control and diabetic male albino rats. Mean ± SD

Physiological condition of animal	Blood glucose level[1]	DNA[2]		RNA[3]	
		Submax. gland	parotid gland	Submax. gland	parotid parotid
Normal	127.85 ± 9.15	0.431 ± 0.010	0.161 ± 0.014	0.246 ± 0.012	0.149 ± 0.018
Control	125.15 ± 12.55	0.412 ± 0.004	0.148 ± 0.004	0.250 ± 0.007	0.152 ± 0.012
Diabetic	257.98 ± 15.85	0.462 ± 0.007	0.182 ± 0.006	0.267 ± 0.018	0.133 ± 0.002
Significant (*P) at the level	P<0.001	P<0.05	P<0.02	NS	NS

1. mg glucose/100 ml blood (as an index of diabetic condition)

2. mg DNA/100 mg fresh gland

3. mg RNA/100 mg fresh gland

*P values refer to differences between normal and diabetic conditions

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

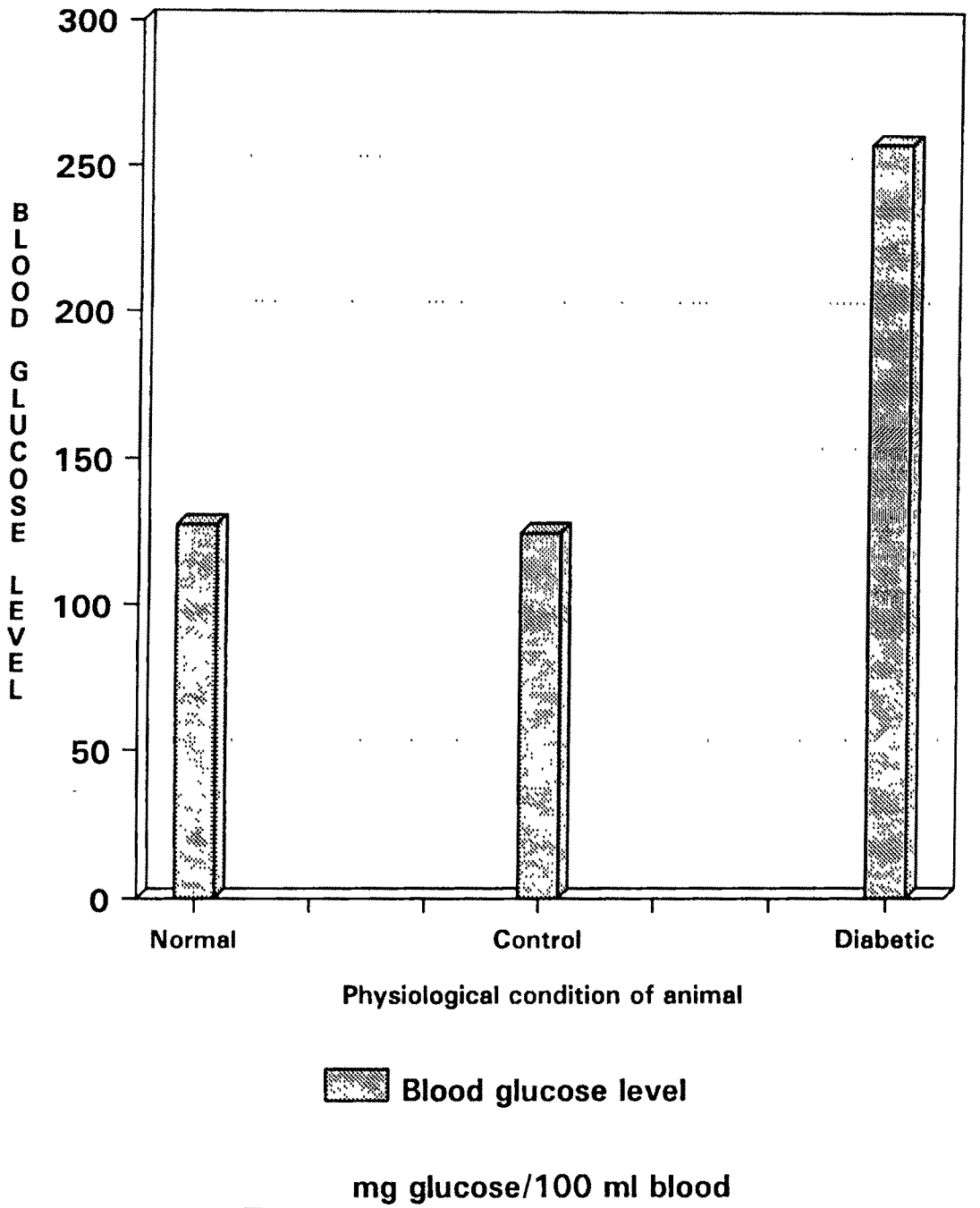

Fig.IIIa. Graphic presentation of Blood Glucose Level of normal, control and diabetic male albino rats.

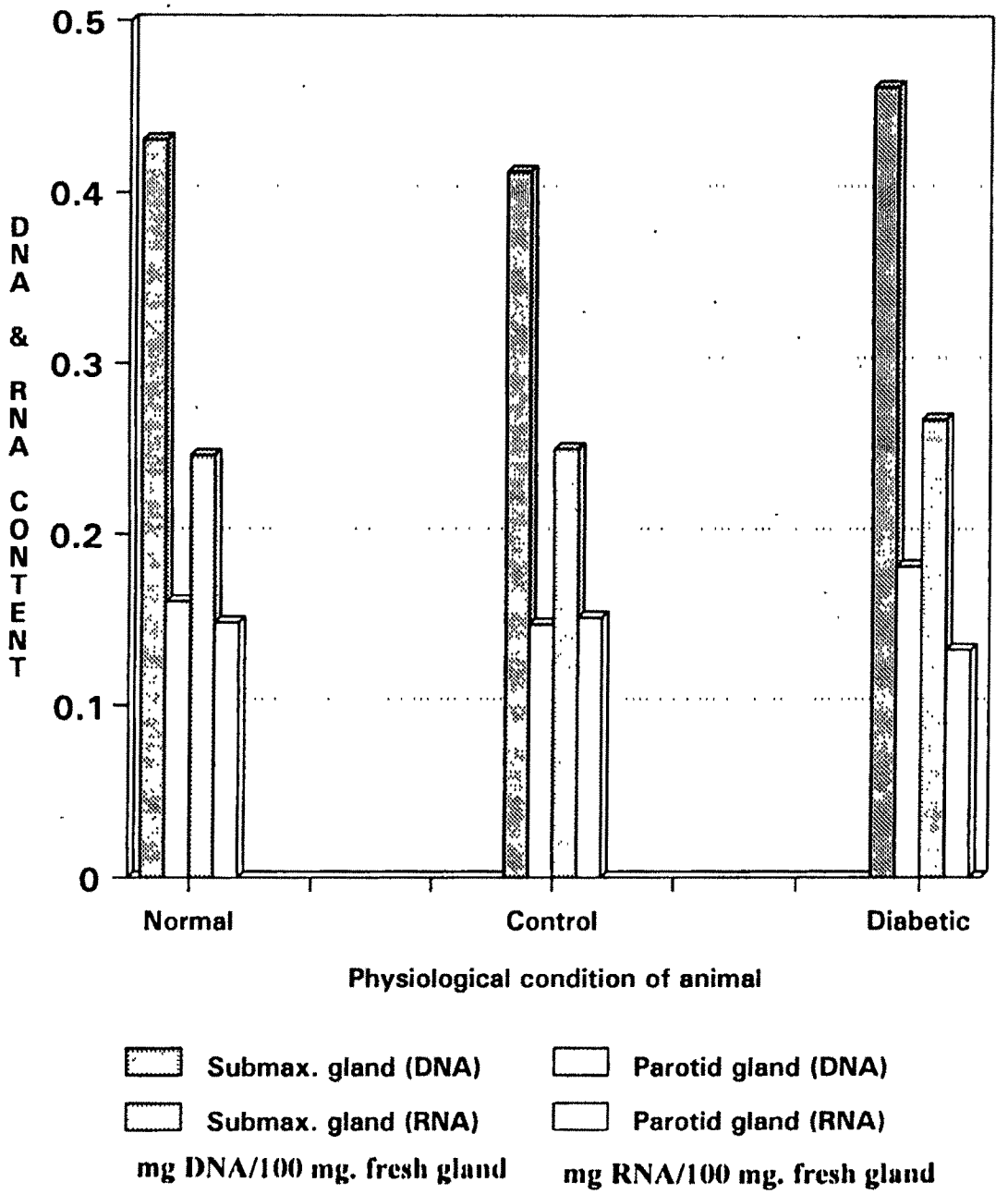

Fig.IIIb. Graphic presentation of Nucleic Acids content (DNA and RNA) in submaxillary and parotid glands of normal, control and diabetic male albino rats.

contents in the diabetic condition, whereas these two types of salivary glands showed non significant and non parallel alterations in their RNA contents. Anderson and Johnson (1981) have observed non significant higher values of DNA and RNA only in parotid gland of diabetic rats in which this endocrine disorder was induced by a single intra-peritoneal dose of alloxan. But the result of present investigation indicates significant higher value of DNA in both the types of salivary glands in diabetic condition. The large number of mitotic figures observed in the parotid glands of diabetic rat is a characteristic of increased functions. Increasing mastication could be one of the factors or reason which result in an increase in gland weight, RNA, DNA and mitotic index (Schneyer and Hall, 1976). It must be noted that gland weight (Chapter I) did not increase in diabetic animals compared with their respective controls. The effects of increased mastication may have been masked by some other aspect of the diabetic state. Again when semisolid food is provided in form of paste of ground ingredients more and hard mastication is not required. Hence, role of more mastication is not important in present investigation. Alternatively, the increased number of mitotic figures could be related to repair processes in the glands also. Such increased mitotic figures have been seen following the thioinine induced acinar cell injury by Leeb (1975) which was not due to mastication. At the same time, it would be necessary to mention that not only both the glands failed to increase in weight but also parallel decrease in their glycogen and lipids contents (Chapter II). This fact indicates that glands become smaller or got shrunk in diabetic condition. Therefore values of stored metabolites (glycogen, lipid etc) showed apparent higher values which is further magnified by their accumulation due to decreased utilization in insulin deficiency. In such circumstances alterations in nucleo-cytoplasmic ratio is also expected in endocrine disorder. Thus an increased DNA contents of these glands may be misleading rather than an effect.

From the higher percentage of alterations in parotid it could be stated that pure serous type parotid gland is more sensitive than the submaxillary which is a mixed type. RNA is entirely different from DNA but all the types of RNA have been synthesized from DNA. Generally alterations of these two nucleic acids show parallel or identical responses. The final result of insulin stimulation of cellular activity is the synthesis of macromolecules of glycogen, lipid, protein, and nucleic acid. Explanation for this generalized anabolic action of insulin can be derived from the well known fact that most of the cellular metabolic activities arise from and is regulated by structural and enzymatic protein and that regeneration of protein is

ultimately controlled by nucleic acid synthesis. Insulin is well known for its growth promoting action in general (Gey and Thalhimer, 1924). There have been several reports suggesting the positive influence of insulin on DNA and RNA synthesis (Steiner, 1966) cell division, (Higgins and Anderson, 1931; Bucher *et al.*, 1969; Bucher and Swaffield, 1975 a, b; Bucher and Weir; 1976; Price, 1976) and protein synthesis (Gelehrter and Tomkins, 1970; Reel *et al.*, 1970; Wool *et al.*, 1968, 1972). Karl and Gerald, (1971) have reported marked reduction in protein synthesis in ribosomes in liver cells of diabetic animals. A fall in cellular RNA as well as protein content in diabetes has been reported many years back by Manchester (1967) and Wool *et al.*, (1968). Leslie (1952) has reported that insulin stimulates the synthesis of RNA and DNA by chick heart explants from thirteen day old embryos. Leslie *et al.* (1957), found that insulin increases synthesis of RNA in human skin fibroblasts and kidney cells in tissue culture. Carruthers and Winegrad (1962) reported that insulin markedly potentiates the incorporation of radioactivity from 14_C glucose into adipose tissue RNA.

Anderson and Johnson (1981), have observed non-significant and minor alterations in parotid salivary gland of diabetic animals. In present investigation both the nucleic acids showed non-parallel changes, and more interestingly, different from the observations of Anderson and Johnson (1981). Submaxillary glands showed non-significant higher values, Whereas parotid gland showed non-significant lower value of RNA content in diabetic condition. Considering the stimulatory role of insulin in RNA and protein syntheses and from the significantly lower activities of amylase (Chapter I), it could be observed here that deficiency of insulin has affected RNA content of parotid gland which is pure serous type. Submaxillary gland on the other hand inspite of having higher content of RNA than parotid in normal condition, did not show lower value but showed non-significant higher value. This might be due to the possibility that, mucous acini are not much affected in insulin deficiency.

From the results of present work it could be concluded that levels of both the nucleic acids i.e. DNA & RNA is comparatively higher in submaxillary than parotid in normal condition in presence of insulin. More involvement of nucleic acids in mucous and serous type of acini of submaxillary gland may be the reason for this

higher level. Increased level of DNA in both the glands of diabetic rats seems to be misleading and apparent rather than actual effect of diabetes. Nonsignificant and non-parallel alterations of RNA content of these two glands indicate that function of parotid is comparatively more affected due to deficiency of insulin.