CHAPTER FOUR

DISCUSSION

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In the present study we have used word juvenile adult to the mice of 2 months age only because submandibular gland was removed when mice were juvenile, and they were allowed to grow in sialoadenectomised condition for one month by the time they had become adult.

In sialoadenectomised mice blood glucose level was decreased in juvenile adult (2 months) and adult mice (3 months). The decrease in blood glucose level might have been caused due to removal of submandibular gland indicating the presencee of hyperglycemic factor in it. To impart hyperglycemic state is the function of glucagon (Grodsky, 1973; Unger and Orci, 1976 and El-Refai and Bergman, 1979). Glucagon is mainly secreted by α-cells of islets of Longerhans in pancreas. Silverman and Dunbar (1974); Dunbar _et al._ (1977); Bhathena _et al._ (1977); Kelly _et al._ (1977); Lawrence _et al._ (1976, 1977); Hojvat _et al._ (1977); Hoshino _et al._ (1976); Nishini _et al._ (1981); Perezcastillo and Blazquez (1980);& Smith _et al._ (1979) studied possibility of presence of glucagon like substance in the submandibular gland. Presence of glucagon like substance in submandibular gland is also proposed by Penhos (1975) in totally eviscerated rats. The extracts of the submandibular gland (Sliverman & Dunbar, 1974) or homologus graft (Pisanty _et al._, 1975) as well as glucagon extract from gland (Bathena _et al._.,

1977; Dunbar et al.,1977; Lawrence et al., 1977) caused significantly hyperglycemia in normal rat. But according to Smith et al. (1979) that rat submandibular gland does not contribute to plasma level of immunoreactive glucagon (IRG), nor does it significantly affect the handling of the carbohydrate in intact or eviscerated animals. However, Tahara et al. (1983) thought that the fictitious nature of glucagen in submandibular gland is due to degradation of (^{125}I) glucagon during radioimmunoessay. Large quantity of glucagon like substance was extracted by acid saline method from saalivary gland (Bhathena et al., 1977; Hojvat et al., 1977; Dunbar et al., 1977; Lawrence et al., 1977; Perez and Blazquez, 1980). When (125 I) glucagon is added to the gel filtered submandibular gland acid saline extract, was completely damaged during incubation (Tahara et al.,1985). Immunohistochemical studies have not yet succeeded in demonstrating clearly the presence of glucagon containing cells in salivary glands. In their experiment Tahara and his associates (unpublished work) could not be able to show glycogenolytic activity in liver. These facts seem to deny the existence of immunoreactive glucagon in submandibular gland. According to Tahara et al. (1985) that the possibility of that hyperglycemic effect of intravenously injected AS extract in rats (Bhathena et al., 1977; Dunbar et al..,1977; Hojvat, 1977; Lawrence et al., 1977) and hypoglycemic effect of salivary duct ligation in

genetically diabetic mice (Hoshino _et al._, 1976) may related to proteases present in the granular convoluted tubules of submandibular gland, but this has not been investigated so far and no one who studied salivary gland secretion, its chemistry and effect will agree with this assumption.

Our earlier observation also showed non-significant alteration in the liver glycogen in sialoadenectomised mice. Effect of glucagon on carbohydrate metabolism is to increase the breakdown of liver glycogen to glucose and hence hyperglycemia, but it does not cause the breakdown of muscle glycogen and it has no effect on muscle phosphorylase. Next to liver skeletal muscles are rich source of glycogen. To find out the possible effect of submandibular glucagon skeletal muscles were selected. Skeletal muscles have thhree different types of fibres which are classified as slow twitch oxidative, fast twich oxidative glycolytic and fast twich oxidatve glycolytic (Peter _et al._, 1972). Soleus is red muscle 100 % slow twich aerobic fibres. Rectus abdominus (glycolytic) and gastrocnemius (aerobic glycolytic fast twich muscles. Number of studies have examined glycogen and/or its regulatory enzymes in different muscle types (Peter _et al._, 1972; Terjung _et al._, 1974; Talor _et al._., 1975; Burchell _et al._, 1976; Conlee _et al._, 1976; Fell and Karlson, 1977; Tsutou _et al._, 1985).According to Terjung _et al_ (1974), slow muscle use a lowest amount of glycogen per single twich. Lower level of glycogen catabolic enzyme activities and higher level

of glycogen anabolic enzyme activities in slow twich muscular may serve the prevention of glycogen depletion. The fact also that the glycogen resynthesis after exercise is faster in slow muscle than fast muscle. Tsutou et al. (1985) showed that activities of glycogen synthase (total) and branching enzymes in slow muscles are higher than those of the fast muscles while phosphorylase kinase, phosphorylase and debranching enzymes are reversed.

Effect of salivary secretion on glycogen content of skeletal muscles was studied to augment the effect of salivary glucagon. Earlier studies failed to demonstrate possibility of glucagon in salivary gland mainly because they could not find glycogenolysis in the liver. This may be because of two reason, one of it is already described Tahara et al. (1985), according to them that during isolation of glucagon there may be possibility of its degradation. There is no effect of pancreatic glucagon on muscle glycogen and perhaps salivary glucagon may not be having effect on liver glycogen. This indicates the possibility of two different species of glucagon. Pancreatic glucagon may not be having receptors on muscle cell and for salivary glucagon on liver. Salivary glucagon might be synthesised and secreted by convoluted granular tubules of submandibular gland. Large number of polypeptides well documented, studied and their presence is proved in the convoluted granular tubules of submandibular

gland. Extensively studied structure and properties are nerve growth factor and epidermal growth factors (Barka, 1980; Murphy et al., 1980). These factors are in higher concentration in males than female, rise after androgen treatment or fall following archidectomy. Additionally these substances are present in low concentration or at negligible level in prepubertal gland and that rise in concentration in parallel with the cytoplasmic differentiation of GCT cells. Glucagon may also be behaving similar fashion. Therefore at the age of one month when GCT cells are not fully developed, submandibular glands were removed and also from adult mice. It has been observed that there were significant alterations in the muscles of juvenile adult sialoadenectomised mice and are remarkable in adult sialoadenectomised mice.

Glycogen content was maximum in the soleus muscle which is slow twich muscle. In soleus muscle of juvenile it was low and increased in adult. In the muscle of sialoadenectomised mice it was increased. In the juvenile adult the increase was one and half fold, whereas in aduult it was two fold. Rectus abdominus is white and high twich muscle, whereas gastrochemicals is mixed muscle formed of white and red fibres. In both these muscle glycogen content was more or less same, but behaviour of these muscles as glycogen storage is concerned was like soleus muscle, as in juvenile adult it was low and in adult it was increased, in sialoadenectomised mice in both the cases

glycogen content was increased, though increase of glycogen in these muscle was same and significant it was not that remarkable as noticed in soleus muscles.

Compared to juveniles protein content was increased in adult muscles and it was maximum in rectus abdominum. In both the age groups of sialoadenectomised mice protein content was increased compared to their controls. Regarding alkaline phosphatase the maximum activity was observed in soleus muscles. Compared to juveniles the activity was increased in adult. In sialoadenectomised mice alkaline phosphatase activity was increased. In other two muscles alkaline phosphatase activity was low compared to soleus, but pattern of increase in alkaline phosphatase activity was same as that of soleus. Cohen (1982) and Ingebristen (1983) showed that the protein phosphatase is responsible for the inhibition of glycogenolysis and stimulation of glycogen synthesis. Tsutosus et al. (1985) in their study showed that activities of C-Amp dependent protein kinase and protein phosphatase in slow muscle (soleus) are higher than fast muscles. There are atleast four types of protein phosphatase in skeletal muscles (Cohen, 1982) and 2 times more phosphatase activity in slow muscles than fast muscles. In the present investigation activity was three fold in soleus than the other two fast twich glycolytic and fast twich aerobic glycolytic muscle. presence and behaviour of alkaline phosphatase in the

muscles of sialoadenectomised mice can be related with protein phosphatase, protein phosphatase inhibit glycogenolysis. Increase in alkaline phosphatase can be related with inhibition of glycogenolysis and high level of glycogen in the muscles of sialoadenectomised mice.

Regarding the lactate dehydrogenase activity there was significant decrease in the muscles of sialoadenectomised mice. Lactate dehydrogenase is the regulatory enzyme composed of subunits. Substrate saturation curves under certain conditions are sigmoid (Umbarger, 1964). LDH was separated into five bands. The 4th and 5th bands were at the origin. In juvenile adult I, II, III were clearly separated and IV and V bands were closely associated but could be recognised. In the muscles of sialoadenectomised mice 4th band was not separated. In adult LDH could separated into five bands, and in sialoadenectomised mice it was separated into 4 bands. These observations clearly indicated that in sialoadenectomised M type LDHs are affected. As we know LDH 4 is formed $H_1 M_3$ subunits and LDH 5 is formed of M_4 subunits. Metabolic effects of glucagon are studied by several workers in rat (Rowe, 1970; Schumer, 1973) dog (Vander & Reynolds, 1969; Manchester et al., 1969; Proctor et al.,1980) and pig (indberg & Darle, 1977). Overall, there was lactate extraction, where blood lactate level was increased. In the hyperlactamia there was conversion of pyruvate to lactate, the

reaction is catalysed by lactate dehydrogenase. In the muscles of sialoadenectomised mice low lactate dehydrogenase activity demonstrates the necessity of salivary secretion for carbohydrate metabolism. Low level of lactate dehydrogenase in muscles indicate lack of glucagon due to the removal of submandibular glands. This indicate the presence of glucagen in submandibular gland which affects the carbohydrate metabolism of muscles.

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