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I) Morphology Distribution and Functions of Submandibular gland:

Most of the studies on salivary glands are directed towards the secretion and distribution of protein, enzymes and their hormonal regulation, electrolyte distribution and its influence on the transport phenomenon of electrolytes and their hormonal and nervous regulations and effects of various drugs on their secretion. In recent years since the discovery of biologically active polypeptides in the submandibular gland and their regulation by varioius hormones, the attention has been shifted to study the effect of submandibular gland secretion on other organs as well as on homoeostasis.

1) Occurrence :

Glands which are situated at the anterior portion of the digestive tract and empty their secretion into the anterior portion of the digestive tract are known as salivary glands.

Major salivary glands lie at some distance from the oral mucosa with which they communicate through one or a few extra glandular ducts, and the minor glands, which lie in the submucosa and open through numerous short excretory ducts.

The largest salivary structure is paired parotid glands, each is located near the angle of the jaw and the ear. The submandibular gland lies on either side of the midline are the most conspicuous structures in the ventral cervical region. The major sublingual glands are applied to the antero-lateral surface of the submandibular glands. In all animals nerves from both the divisions of the autonomic nervous system, para sympathetic and sympathetic can be found going to all three great salivary glands (Langley, 1878; Langle and Fletcher, 1889).

2) Microscopic and Ultrastructure of Submandibular Gland :

This gland has well defined capsules and fairly predominant duct system (Davis and Davies, 1962). The most proximal unit of the submandibular gland is the acinus composed of large pyramidal cells grouped around a small lumen. Each acinus is connected by short tubules, intercalated ducts. The intercalated ducts are followed by granular ducts which are also called as granular convoluted tubules.

Granular convoluted tubules are followed by less numerous system of tubules, the striated ducts, which in turn leads into a lesser number of small excretory ducts. Finally small excretory ducts merge into one large excretory duct that leads from main glandular mass to the oral cavity, where it terminates (Leeson, 1967).

Granular ducts have long been interesting because of number of unusual characteristics. These ducts have been considered to be the site of formation of many organic substances like secretory enzymes (Chretien and Zajdela, 1965; Smith et al., 1971; Smith and Frommer, 1972 a,b). The granular ducts also considered as source of salivary kallikrein (Orstavik et al., 1975; Hojima et al.,1977) and non-specific proteases (Shafer et al., 1959; Sreebny and Meyer, 1964; Riekkinen and Niemi,1968; Bhoola et al., 1973). It has also been suggested that granular ducts are the source of renin (Bing and Farup, 1965; Bing et al., 1967; Bhoola et al., 1973; Gutman et al., 1973) and nerve growth factor (Goldstein and Burdman, 1965; Hendry and Iverson, 1973; Schwab et al., 1976) epidermal growth factor (Cohen, 1962) and various mesodermal growth factors (Weimer and Haraguchi, 1975).

3) Process of Secretion :

Secretory cells have been endowed with double duty of synthesizing protein and glycoprotein not only for internal need but to carry out funcctions extracellularly. The secretion of protein or glycoprotein, at least in some degree, may be characteristic of all cells. Glandular tissue in higher animals has become specialized in this function. The problem that is solved by the secretory cell is the sorting out of proteins and glycoproteins destined for secretion from those which are used

intracellularly. The complexity of this problem is solved in number of reviews (Blobel et al., 1979; Blobel, 1980; Davis and Tai, 1980).

Incident sequence of events in the course of synthesis, concentration and packing of secretory products are studied by Castle et al., (1972) in the rabbit parotid gland, using some method and the process of glycoprotein synthesis in the mucous cells have been investigated by Neutra & Leblond (1966a,b), Bennett et al., (1974). According to these workers proteins are synthesised on the polysomes then transported through cisternal lumina of the rough endoplasmic reticulum to Golgi systems via transitional vesicles (Castle et al., 1972). In salivary glycoprotein secreting cells, N-acetyl-galactosamine and mannose sugars are added at the serine or threonine of polypeptide by means of glucosyl transferases (Young and Van Lennep, 1978). Addition of remaining sugars are thought to occur mainly in the Golgi complex and probably also in the transitional vesicles. Studies with radioactive labelled fucose marker's (Bannett and Leblond, 1970; Bennett et al., 1974) have shown that 3H-fucose incarporation into polypeptide chain takes place within the Golgi complex of glycoprotein secreting cells.

The membrane of this organelle contains necessary glycosyl transferase (Schachter, 1974). The process of condensation of glycoprotein seems to start in Golgi complex (Jamieson

and Palade, 1971). There are several papers emphasizing the possible role of the Golgi Endoplasmic Reticulum Lysosome (GERL), in the processing and packing of secretory proteins into secretory granules (Novikoff, 1976; Hand and Oliver, 1977a,b; Novikoff and Novikoff, 1977). GERL is defined as special part of the smooth endoplasmic reticulum, generally situated on the innerside of Golgi complex proper.

During exocytosis the granule membrane fuses with the plasma membrane thus increasing the apparent surface area of the plasma membrane. This excess membrane is removed by reabsorption. Reabsorption of the membrane may almost certainly take place by endocytosis (Abrahams and Holtzoman, 1973; Geuze and Poort, 1973; Orci et al., 1973 and Kramer and Geuze, 1974).

Excessive secretory granules get digested in lysosomes. The lysosomes presumably first fused with granules and subsequently digested them in lysosomes (Van Lennep et al.,1977).

The above brief review on the synthesis of secretory material in the salivary gland indicated that - a) The process of protein and glycoprotein synthesis is mainly accomplished by RER and Golgi complex and packing of secretory material takes place at Golgi Complex and GERL. b) Glycoproteins and proteins from salivary gland are secreted by way of exocytosis. Unsecreted granules and secretory granule membranes are absorbed into the lysosomes. Thus lysosomes are playing active role in

the process of secretion.

This is the novel pathway of salivary secretion, but the existence of several biologically active factors in the saliva and blood leads inevitably to the question whether the gland is the source of these factors in the plasma and whether submandibular gland function as an endocrine gland. By following the classical, physiological route of ablation this question could not be answered unequivocally, but the effect of ablation of the gland on the physiology of other organ will help to establish the role of submandibular gland secretion on the physiology of other organs.

4) Functions of Salivary Glands :

Salivary glands can be considered as subserving four types of functions. First and perhaps most important, they evidently provide lubrication to aid the swalloing of food. The lubricant may take the form of slimy mucus, as is usual in lower vertebrates, but more serous secretion, as are formed by the parotid glands of mammals, clearly achieve the same purpose when the ingested food is very dry (e.g. in ruminants). Included under the heading of lubrication is the further, obvious function of keeping the buccal cavity moist and clean with the implied effects that this must have an appettie; in man, moistening of the surfaces of the buccal mucosa seems also be necessary for speech. According to Ellison (1964) submaxillary

was found to contain a great variety of carbohydrates. Parotid and submaxillary saliva are found to contain both non-dialysable and dialysable glucose, while non-dialysable fucose, sialic acid hexasamines are present in parotid, but both non-dialysable and dialysable hexosamine and sialic acid are present in submaxillary saliva.

Second salivary glands, by secreting enzymes, are able to play some role in digestion. Most of the animals have relatively high concentrations of amylase in their parotid saliva and somewhat lower concentrations in the mandibular secretions, although certain species of bat and the common laboratory white rat, have almost no amylase in their mandibular saliva, and in both the parotid and mandibular saliva of the domestic cat and dog, the concentrations of this enzyme are rather low (Chauncey and Quintarelli, 1961; Junqueira and Fava-De-Morues, 1965). Although in most aimals there is no doubt that, given time, salivary amylase can digest a substantial part of ingested starch.

A third role for salivary glands can be discerned in fur-bearing animals such as the cat and the rat, where the animals wet their fur with saliva in response to heat stress, thereby obtaining the same cooling possibility available to man by sweating (Hainsworth and Stricker, 1970). Another function, in some cases is that of defence and of killing or paralysing prey.

A fourth rule for salivary gland is production of pharmacologically active compounds. During the past few decades the number of biologically active polypeptides have been isolated from or claimed to be present in the submandibular glands.

5) Major Polypeptides in Submandibular Gland :

The chemistry and biology of several submandibular gland factors have been extensively studied, however, significance of presence of these factors in the salivary gland has not been worked out in detail except few like nerve growth factor and epidermal growth factor.

The submandibular gland factors operationally grouped into (1) growth and differentiation, (2) Homeostasis, (3) Intracellular regulation and (4) Digestion. The concentration of these factors in the gland of neonatal animal is very low, with onset of puberty concentration of these factors increases very rapdily. Granular convoluted tubules are the site of formation of these substances. Differentiation of granular convoluted cells is hormone dependent (Young, 1980, Barka, 1980).

i) Nerve Growth Factor (NGF):

Johanson et al., (1971), Hendry (1972) showed that nerve growth factor is of submandibular gland origin. The structure of NGF is presented by (Young et al.,1978; Young

1978) according to them NGF occurs in the submandibular gland in multiple molecular forms; the only stable form has a molecular weight of 116,000. This stable form is claimed to be secreted in large quantities into the saliva (Aloe and Levi-Montalcini, 1980, Ances, 1973).

Immunocytochemical staining established unequivocally that NGF is localized in the secretory granules of the GCT Cells (Goldstein and Burdman, 1965; Kumar et al., 1972; Schwab et al., 1976; Simson et al., 1978). Levi-Montalcini (1960) Caramia (1966), and Ishii and Shooter (1975) suggested that NGF was not only stored but also synthesized in the submandibular gland. The concentration of nerve growth factor in the glands of neonatal mice is very low but apparently measurable with the onset of the puberty, NGF concentration increases rapidly (Levi-montalcini et al.,1960, Caramie et al..,1962 and Hendry et al. 1972).

Submandibular gland saliva contains high concentrations of NGF and that the secretion is mediated primarily by alpha-adrenergic mechanisms (Wallace & Partlow, 1976; Wallace et al., 1977; Simson et al., 1978; Hirata and Orth, 1979 , and Murphy et al.,1980). The plasma NGF level reduced by sialoadenectomy return to normal (Hendry & Iversen, 1973), would support a multifocal origin of this factor (Murphy et al., 1980).

ii) Epidermal Growth Factor (EGF):

Next to NGF, epidermal growthfactor (EGF) is the best characterized and most extensively studied, factor that occurs in the sumandibular gland. It was discovered by (Cchen, 1962) as the principle in extracts of male mouse submandibular gland. Epidermal growth factor is a polypeptide isolated from the submaxillary glands of the male mouse Cohen (1962), which exhibits growth stimulating activity on various epidermal and epithelial tissues, both in vivo and in vitro.

EGF is a single polypeptide having aspargine at the NH_2-terminus, arginine at the COOH-terminus, and a tool of 53 amino acid residues (Taylor et al.,1970; Taylor et al., 1974). It contains 6 half-Cystinyl residues and no detectable free sulfhydryl groups. It is further characterized by the absence of three amino acids : lysine, alanine, and phenylalanine. A human epidermal growth factor immunologically related to mouse EGF, has also been isolated.

A human epidermal growth factor, immunologically related to mouse EGF, has been isolated (Cohen and Carpenter, 1975; Starkey et al., 1976). EGF stimulates the growth of epithelial cells, fibroblasts and glial ceells under various experimental conditions (Cohen, 1960, 1962;Jones et al.,1966; Turkington, 1969 a,b; Cohen & Taylor, 1974, Cohen and Savage et al.., 1974; Cohen et al.., 1975; Lembach, 1976; Carpenter & Cohen, 1976; Westermark, 1976 and Tadara et al.,1976).

Tadara et al., 1976; Turkington, 1969a,b; Westermark, 1976).

A large body of evidence indicates that EGF is synthesized, stored and secreted by granular convoluted tubule (GCT) cells of the submandibular gland, and that the concentration of EGF in the gland is closely correlated with the development and differentiation of the GCT under different physiological and experimental conditions. By using an immunofluorescence technique. Turkington et al. (1971) localized EGF primarily in the basal cytoplasm of all GCT cells. In contrast, on the basis of light microscopic immunoperoxidase staining, Cohen and Savage (1974) described that EGF is concentrated in the apical secretory granules of he GCT cells. In crude homogenates of mouse submandibular gland EGF occurs as a high molecular weight (74,000) ccomplex, consisting of two moles of EGF and two moles of an EGF-binding protein.

EGF is first detectable in the gland around the 20th postnatal day. Immunocytochemically, EGF was first demonstrable in scattered GCT cells at 20 days of age in male and at 30 days of age in female mice (Gresik , 1978). The saliva of the male mouse contains far more EGF than the saliva of the female animal (Hirata and Orth, 1979). After puberty concentration of EGF increases rapidly(Byyny et al., 1972), but maximal levels are not reached even at three month of age.

The male mouse submandibular gland contains 1-2 µg/mg (wet weight) of EGF. The level of EGF in the gland is androgen-dependent. The gland of the female contains about 1/10 or less of that of the male. Castration reduces, while administration of testosterone to female or castrated male increases EGF concentration in the gland (Roberts, 1974; Frati et al., 1976; Hojima et al., 1977; Ladda, 1979; Hirata and Orth, 1979).

EGF is a potent mitotic stimulant for a variety of cell types; it enhances Keratinization, and inhibits gastric acid secretion. It is widely used experimentally, not only in investigations of regulation of cell replication, but also as a convenient tool for the analysis of receptor hormone interactions, and receptor-mediated endocytosis of hormones.

Attardi et al., (1965) purified mesodermal growth factor from mouse submandibular gland that caused dedifferentiation of muscle fibers (loss of myosin) and cartilage, and stimulated the growth of mesenchymal cells. This factor displayed protease and esterase like activities and these activities are androgen dependent (Weimer and Haraguchi, 1974)5

iii) Renin :

Cohen and his Coworkers (1972) obtained two renin like enzymes from the subandibular glands of male mice in pure and stable forms. The submandibular gland of the male mouse contains

20 to 30 fold more renin than the gland of the female (Trautschold et al., 1966; Michelakis et al., 1974; Hirata and Orth, 1979). Renin is an acidic protease (peptidase) with a highly restricted substrate specificity. Renin is important in blood pressure regulation, since it catalyzes the first step in the renin-angiotensin-aldosterone cascade. Bing et al. (1977) showed that aggressive behaviour in mice resulted in a vast (upto 600-fold) increase in plasma renin level, which was unaffected by bilateral nephrectomy but was greatly diminished by sialoadenectomy, support the contention that the submandibular gland is a source of renin in the plasma,Bing and Poulsen (1977).

The submandibular gland of the male mouse contains 20 to 30 fold more renin than the gland of the female (Trautschold et al., 1966; Michelakis et al., 1974;Hirata and Orth,1979). The androgen dependency of renin was indicated by the decrease of its level following castration and by its induction by testosterone (Trautschold et al.,1966; Bhoola and Dorey,1972; de Jong et al.,1972; Michelakis et al., 1974; Gecse et al., 1976).

Secretion of renin into saliva is stimulated by alpha adrenergic mechanisms (Manzie et al.,1974; Bing, et al..,1977). The assessment of the contribution of submandibular gland to the maintenance of the plasma renin level is complicated by the apparently unavoidable, artifactual long-lasting release of renin from the gland by sialoadenectomy, or even by gentle manipula-

tions (Bing et al., 1976, 1977; Bing and Poulsen, 1977).

iv) Kallikrein :

Kallikreins have been found in, and isolated from, the submandibular glands of many species man, dog, cow, horse, cat, rabbit, mouse, rat hamster, guinea pig (Werle et al.,1936; Hopsu-Havu et al., 1967; Bhoola et al., 1972; Nustad et al., 1974; Brandtzag et al., 1976; Moriwaki et al.,1976; Proud et al., 1977; Fukaka et al., 1979; Lemon et al., 1979; Maltra et al., 1986). Of all tissues, the rat submandibular gland contains the highest concentration of Kallikrein Erodos et al., (1968).

Garrett (1982) showed that Kallikrein-like activity in human salivary glands. Compared to tche submandibular gland the concentrations of Kallikrein in the sublingual and parotid glands are negligible; Werle et al.,(1936). With respect to Kallikrein content, the submandibular glands of rat and mouse show no significant sexual differences, and neither casteration nor testosterone treatment had a significant effect on Kallikrein levels (Bhoola et al., 1974; Gecse et al., 1976). According to Orstavik et al., (1975) Kallikrein specific immunofluorescence staining was seen as a luminal rim in the intralobular ducts in both the sublingual and submandibular glands of rat at the age of one week. An age-related increase in Kallikrein content in both rat and mouse submandibular gland has been interpreted

erroneously as an indicator of acinar cell localization of Kallikrein since it coincided with differentiation of acinar cells (Gautvik et al., 1974; Nustad et al., 1974).

v) Glucagon :

A) Occurrence :

Glucagon is secreted by the alpha cells of the islets of Langerhans of the pancreas. Glucagon is a small protein and comprises of twentynine amino acids. It occurs in a straight-chain configuration and has a molecular weight of 3485 dalton. Histidine is the N-terminal and threonine is the C-terminal amino acid. Glucagon is relatively insoluble in water but retains potency in acid and basic solutions. Integrity of the molecule is necessary for its biological activity.

A substance similar to pancreatic glucagon in immuno-reactivity is secreted by the gastric, duodenal mucosa (C.C.Chattarjee) and has been named gut Glucagon-like immuno-reactive material (GLI). It is not exactly identical to pancreatic glucagon immunologically. Its molecular weight is approximately twice that of the pancreatic glucagon. The main difference between the two peptides is that GLI is not hyperglycemic in its action. Its secretion is increased by many substances including glucose, which causes an apparent elevation of total glucagon in circulation. Therefore, it is imperative that a pancreatic glucagon specific antisera is used for the radio-immunoassay of glucagon.

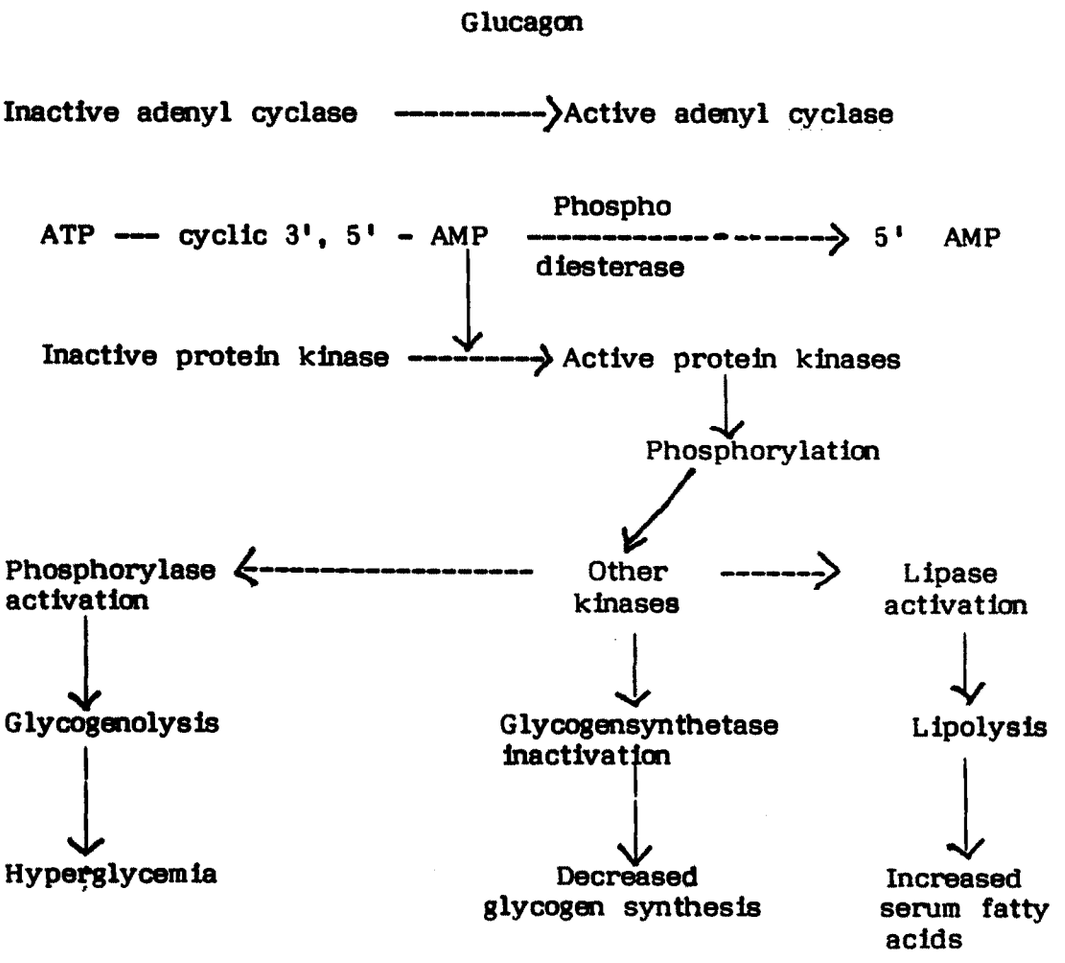
Silverman and Dunbar (1974) first described glucagon in extracts of rat submandibular gland, and showed that such extracts increased blood glucose level in intact rats but not in eviscerated rats. They suggested that the submandibular gland participates in the entero-insulin axis by secreting glucagon, which in turn stimulates insulin secretion. The presence of glucagon in the submandibular glands of several species including the mouse, rat, rabbit, guinea pig, dog and man has been confirmed by Lawrence et al (1975, 1976, 1977); Dunbar et al. (1977); Hojvat et al. (1977).

In contrast to the submandibular gland, only insignificant amount of Glucagon were found in the parotid and sublingual glands (Lawrence et al., 1975, 1976, 1977). According to Kelly et al., (1977) the glands of male rats contain about three times more glucagon than the glands of female animals. The existence of immunoreactive glucagon in salivary glands has long been accepted, and this salivary gland glucagon has been reported to be a hyperglycemic factor with a molecular weight much larger than the pancreatic glucagon, (Dunbar et al., 1977; Hojvat et al., 1977; Lawrence et al., 1977).

B) Functions of Glucagon :

Glucagon and insulin have opposing functions on glycogenolysis, gluconeogenesis, ureogenesis and ketogenesis. Glycogenolysis and gluconeogenesis are about equally sensitive to

glucagon. Glucagon stimulates glycogenolysis by activation of hepatic phsophorylase and this is mediated by adenosine 3', 5'- (Cyclic) monophosphate (cyclic AMP). Glucagon activates the membrane bound adenyl cyclase enzyme complex responsible for converting ATP into hormonally active cyclic AMP.



Mode of action of pancreatic glucagon.

vi) Insulin :

Insulin is a protein with an isoelectric point of 5.3, consists of two polypeptide chains. A chain and B chain. The three dimensional structure of insulin is related to its biological activity. It contains two polypeptide chains and a total of fiftyone amino acid residues, of which twentyone are present in the A chain which contain one intrachain disulphide bond and the rest thirty-one residues are present in the B chain. The two chains are held together by two interchain disulphide bonds (Gyton, 1981, Talwar et al., 1989).

The insulin plays an important role in storing the excess energy substances. In the case of excess carbohydrates, it causes these to be stored as glycogen mainly in the liver and muscles. It causes fat storage in the adipose tissue. All the exceess carbohydrates that can not be stored as glycogen are converted under the stimulus of insulin into fats and also stored in the adipose tissue.

Insulin promotes the utilization of glucose and lowers blood glucose concentration. This is accomplished by increasing the activities of glycogen synthetase, hexokinase and glucokinase which utilize glucose for glycogen synthesis and phosphorylation.

Insulin also increases the activities of the enzymes that promote glycogen synthesis, including phosphofructokinase, which

causes the second stage in the phosphorylation of the glucose molecule and glycogen synthetase, which is responsible for polymerization of monosaccharide units to form glycogen molecules. The net effect of all these actions is to increase the amount of glycogen in the liver.

Hisatak et al.(1980) demonstrated that insulin promotes activation of glycogen synthase by(^{14}C) glucose incorporation into glycogen in perfused rat skeletal muscle without changing the level of CAMP or altering activation state of CAMP-dependent protein kinase or phosphorylase Chiasson et al. (1980).

Insulin inhibits liver phosphorylase, the enzyme that causes liver glycogen to split into glucose. This obviously prevents breakdown of the glycogen that is already in the liver cells. Insulin causes enhanced uptake of glucose from the blood by the liver cells it is due to increasing the activity of the enzyme glucokinase, which is the enzyme that causes, the initial phosphorylation of glucose after it diffuses into the liver cells.

Insulin can suppress glucagon secretion in vivo and in vitro (Samols et al., 1970, 1972; Samols and Harrison, 1976; Weir et al., 1976). Glucose, itself, in the absence of an appropriate insulin concentration, does not suppress glucagon (Samols et al.,1983; Samols, 1983).

The action of insulin has been ascribed to the generation

of a labile intracellular inhibitor of CAMP binding to the kinase (Walkenbach et al., 1978). Evidence of the existence of this inhibitor in muscle extracts has recently been reported by Walaas et al. (1973). The presence of non-suppressible insulin like material is found by RIA and immunofluorescence staining described and reviewed by Barka (1980).

These biologically active factors though synthesized and secreted by GCT cells of the submandibular gland, their formation is dependent on hormones like androgen, thyroid and probably adrenocorticoid hormones. Extirpation of submandibular glands may lead to an acute shortage of their factors in the plasma, this indicate endocrine like function of submandibular glands. Efforts are carried out to find out effect of these factors on other tissues.

II) Definition, Location, Composition of Muscles :

1) Muscles :

Definition : Muscles are biological machines which convert chemical energy into mechanical work by which animal body can respond suitably to environmental changes. There are main three types of muscles, skeletal, cardiac, visceral.

2) Skeletal muscle (Voluntary or Striated) : These muscles are responsible for voluntary movement of the living system.

These are mostly attached to bones of the tendons. A tendon is composed of densely packed white fibrous (non-elastic) connective tissue. At the junctional point, the fibres of the tendon is affixed to the sarcolemma of the muscle fibres. Muscles are formed of muscle fibres. Muscle fibres are multinucleated cylindrical structures having clear display of longitudinal and cross striations. A muscle fibre being composed of a number of delicate fibrils surrounded by a more fluid sacroplasm and having mitochondria, sarcoplasmic reticulum and possess respiratory pgiment myoglobin (muscle haemoglobin). Red colour of muscle fibre is due to presence of myoglobin. White or pale muscle fibres are deficient in myoglobin.

1) Rectus abdominis :

Rectus abdominis muscle is white muscle. This muscle is a flat thin band extending the whole length of the ventral surface of the trunk from syniphysis pubis to the anterior end of the sternum. It is inserted into the first rib, the medial third of the clavicle and into the manubrium. In the abdominal region it is separated from the corresponding muscle of the opposite side by the linea alba. The muscle of each side arises by two, occasionally three, slips which cross the mid-line alternating with corresponding slips from the rectus abdominis of the opposite side, giving a striking interdigitation.

This muscle help during the expiration evacution and also support the viscera (intestines only).

ii) Gastrocnemius :

It is mixed muscle and made up of two heads, medial and lateral. The medial head arises from the medial epicondyle of the femur and from the medial fabella. The lateral head takes its origin from the lateral epicondyle and from the lateral fabella. Their tendons are twisted with that of plantaris.

The lateral head of gastrocnemius arises from the epiphysis and shaft. The medial head of gastrocnemius arises by a tendon from a smooth shallow pit on the medial condyle at the lower end of the medial supra condylar line.

The gastrocnemius supplies the main propulsive force in walking, running or jumping.

iii) Soleus :

Soleus muscle is a red muscle. It lies beneath the gastrocnemius muscle. Soleus muscle is multipennate muscle. It arises by a slender tendon from the head of the fibula. Both muscles unite in a strong tendon and insert on the tuber calcenei.

It is slow-acting but more powerful than gastrocnemius. In walking, the soleus acts as the first (bottom) gear, and the gastrocnemius as the third (top) gear. The soleus overcomes the inertia of the body weight and when the movement is underway, the quicker action gastrocnemius increases the speed.

3) Composition of Muscles :

The muscle contains 75 % water and 25 % solid.

1) Protein 20 %, fats 0.2 % including cholesterol, lecithin and natural fats.

2) Carbohydrates 1.0 % (Glycogen 0.5-1.0 % and Hexose phosphate 0.05 %).

3) In organic salts 1.0 - 1.5 (Potassium phosphate mainly) and trace of Ca, Na, Mg, Fe, Cl and sulphate.

4) Non-nitrogenous -

1) Lactic acid 0.02 % in fresh resting muscle.

5) Inositol (muscle sugar, hexahydroxy cyclohexane) 0.25 %

6) Nitrogenous - Adenosine triphosphate ATP 0.25 %, Adenosine diphosphate (ADP), Adenosine monophosphate (AMP), Phosphagen 0.5 %.

7) Xanthin, Hypoxanthine and inosinic acid, carnosine 0.3 % and pigments.

8) Pigments - myoglobin - It is an iron containing chromoprotein found in red muscle.

9) Cytochrome - It is an iron porphyrin pigment found in three forms a,b,c, flavins and other. Enzymes and coenzymes of the glycolytic cycle, citric acid cycle.

10) There are several enzymes in high concentration in muscles related with carbohydrate metabolism and concerned with contraction of muscles. Glycogen is stored in muscles as

a ready source of carbohydrate metabolism Levels of enzymes and substances related to carbohydrate metabolism dependent on the levels of glucagon and insulin which are synthesized in pancreas, this fact is well established. But an effect of glucagon and insulin like substances released from submandibular gland on muscle carbohydrate metabolismhas not been discovered so far.

Glycogen :

Glycogen is the reserve carbohydrate in animals. It is in the form of glucose and remains stored in the liver and muscles. It is a highly branched chain polysaccharide consisting of hundreds of glucose units linked together by glucosidic linkages which are made up by the loss of water between hydroxyl groups. The Glycogen is made up of D-glucose residues, upon hydrolysis, it yields, D-glucose as the product. The glucose residues are linked together through α-1, 4-glycosidic linkages except at the branch points. The branch is linked to the main through α-1, 6-glycosidic linkages.

The average molecular weights of glycogen preparations vary from 270,000 to 100,000,000. All cells of the body are capable of storing at least some glycogen but certain cells can store large amounts, especially the liver cells which can store

upto 5 to 8 per cent of their weight as glycogen and muscle cells which can store upto 1 to 3 per cent.

Lower levels of glycogen catabolic enzyme activities and higher levels of glycogen anabolic enzyme activities may serve for prevention of glycogen depletion. The fact that the glycogen resynthesis after exercise is faster in slow muscle than in fast muscle may be due to the high latent capacity of glycogen anabolic enzymes (Terjung et al., 1974). The enzymatic profile of fast muscle seems to be convenient for the short-duration fast contraction and might explain its easy fatiguability and slow glycogen resynthesis after exercise. Fast muscle showed higher glycogen content (Peter et al., 1972; Terjung et al., 1974).

IV) Lactate Dehydrogenase :

The interconversion of lactic acid and Pyruvic acid is catalysed by lactate dehydrogenase, a reaction that is linked to anaerobic metabolism. Under anaerobic conditions, this reaction temporarily takes the place of the citric acid cycle of mitochondria. Both Pyruvic acid and NADH are normally removed from the metabolic pool by the citric acid cycle under aerobic conditions. Under anaerobic conditions, Pyruvic acid is converted to lactic acid with the formation of NAD from NADH. Lactic acid is not further metabolized, but it must be reconverted to Pyruvic acid in another tissue in which Oxygen is available.

Lactate Dehydrogenase (LDH, E.C. 1.1.1.27) is polymeric enzyme, composed of four peptide chains of two types of subunits M & H. Each is under separate genetic control. Thus five isoenzymes of LDH have been observed, differing in the number of H & M units in their active tetrameric forms. The fastest moving electrophoretically is of LDH-1, composed of four H units, the next fastest, is LDH_2 or H_3M followed by LDH_3 or H_2M_2, LDH_4 or HM_3 and LDH_5 or M_4, the slowest band. The M_4 and H_4 lactate dehydrogenase have differing enzymatic properties, which reflect their intended function. The H_4 form is inhibited by very low concentrations of pyruvate, making it useful to tissues that must convert lactic acid to pyruvic acid. The M_4 form is inhibited only by high concentrations of pyruvic acid, making it useful to tissues that have short bursts of anaerobic metabolism.

Because of the difference in behaviour of the H_4 and M_4 forms of lactate dehydrogenase, it is possible to demonstrate the two forms differentially. The ground work for this technique was developed by Brody and Engel (1964) and it was refined by McMillan (1967) and Jacobsen (1969).

Lactate dehydrogenase from tissues which is resolved electrophoretically into five isozymes each of which is a tetramer. These tetramers can be dissociated into monomers by

freezing in 1 M sodium chloride. On thawing, reassociation into functional tetramers occurs. On the basis of change and amino acid composition there are two kinds of monomers. Lactate Dehydrogenase-1 contains one kind of monomer and lactate dehydrogenase-5 the other kind. A mixture of equal quantities of these two isozymes, after dissociation and reassociation, leads to the production of all five isozymes in the expected proportions of 1:4:6:4:1.

Distribution :

It has been clearly established by a variety of different techniques that five distinct isoenzymes usually occur in human and animal tissues, their relative distribution varies considerably, not only from tissue to tissue but also from species to spepcies. In heart, erythrocytes and kidney, the fast moving isoenzymes LD_1 and LD_2 predominate, whereas in liver and skeletal muscle the principle isoenzymes are LD_4 and LD_5. LD_3 appears to be the most abundant fraction in many other human tissues, including the spleen, pancreas, thyroid, adrenals and lymphnodes.

Several investigators have reported the absence of LD_5 from red blood cells. However, it has now been established that erythrocytes lose their complement of this isoenzyme during ageing (Wilkinson, 1970).

Differentiation of the isoenzyme pattern is especially well marked in skeletal muscle, but in progressive muscular dystrophy of the pseudohyper trophic (Duchenne) type such a change remains substantially incomplete and LD_1, LD_2 and LD_3 remain the principal isoenzymes (Shepard et al., 1965).

The total lactate dehydrogenase activity of human dystrophic muscle is also much less than that of normal human muscle, and as the serum activities of enzymes found in muscle (lactate dehydrogenase, aspartate amino transferase, creatine kinase, aldolase) are much increased in progressive muscular dystrophy, it seems that the fall in the lactate dehydrogenase activity of dystrophic muscle may be partly due to an increase in membrane permeability (Zondag, 1963; Brody, 1964; Dawson et al., 1964; Shepard, Gordon and Wollenweber, 1965). The rat red muscle (Soleus) had a (high activity of succinate dehydrogenase) and low activity of lactate dehydrogenase LDH. The isoenzyme pattern of soleus LDH was of heart type (fast components predominated). The white muscle had low SDH and high LDH activities and a LDH isoenzyme pattern of the muscle type (Rezvyakov, 1972).

Male rabbits at various stages after denervation of sciatic nerve were examined by Zamoskouskaya et al. (1974). For enzymic (Gaidadzhiev, 1974) activity in the sarcoplasm of their soleus and gastrocnemius muscles (Gaidadzhiev, 1974).

The activity of lactate dehydrogenase as well as that of NAD- and NADP- specific malate dehydrogenases, was measured during neurogenic atrophy and recovery of motor functions of the muscles and shows that MDH/LDH ratio in the intact soleus muscle was higher than in the gastrocnemius muscle (1.68 and 0.67 resp.) also shows that during max. atrophic changes in muscles (5 weeks after denervation), this ratio was 1.0 in soleus muscle and 1.27 in gastrocnemius muscle. The decrease in NADP-MDH activity in denervated soleus muscle occurred earlier and to a greater degree than in gastrocnemius muscle. The recovery of enzymic activities to normal was accomplished earlier than the restoration of total protein content in muscles and their motor functions. Exercise induce decrease in lactate dehydrogenase activity, and it as more marked in white muscle than red (York et al.,1974; Brinkworth & Masters, 1978; Chin et al.., 1980).

V) Alkaline Phosphatase :

Alkaline phosphatase (orthophosphoric monoester phosphohydrolase EC 3.1.3.1) have been divided into three categories based on kinetics, response to inhibitors, stability, and electrophoretic mobility (Moss, 1969 a). The first type, placental alkaline phosphatase, occurs in the human placenta but generally not in adult tissues. However, tumor tissues sometimes elaborate a kind of alkaline phosphatase that resembles placental alkaline

phosphatase. The second type is intestinal alkaline phosphatase, which also somewhat resembles placental alkaline phosphatase. The third category comprises the alkaline phosphatases, which occur in bone, kidney, and liver. The placental enzyme is clearly under separate genetic control, which differentiates it from the others. Alkaline phosphatases from other sources are fairly heterogeneous, and it is uncertain whether they represent separate genetic groups or whether the differences result from carbohydrate prosthetic groups. Alkaline phosphatase from an osteosarcoma has been shown to be completely different from liver, intestinal, kidney and placental alkaline phosphatase on immunological ground (Singh and Tsang 1975).

Alkaline phosphatase contains zinc as an integral part of the molecule and is therefore, considered a metalloenzyme. Its active centre contains a serine residue, and the mechanism of action involves the formation of phosphoryl serine residues. The enzyme contains a number of carbohydrate prosthetic groups including sialic acid, but the amount varies from one type to another.

High concentrations of alkaline phosphatase are often associated with absorptive cells, especially in the brush borders of the intestinal mucosa and the proximal tubules of the kidney. Alkaline phosphatase is always present wherever calcification occurs, particularly in osteoblast and in chondrocytes of

cartilage that is about to give way to endochondral bone formation.

In addition to being able to hydrolyze a wide variety of monophosphates, alkaline phosphatase is also quite capable of hydrolyzing pyrophosphates (Cox and Griffin, 1965). It has also been reported to hydrolyze ATP (Moss and Walli, 1969).

It has long been knwon that most types of alkaline phosphatases are activated by magnesium ions, but Moss (1969b) showed that only the orthophosphatase activity of alkaline phosphatase is activated; magnesium ions actually inhibit pyrophosphatase activity. Zinc ions inhibit alkaline phosphatase of bone and cartilage (Takada et al., 1968). Highly specific inhibitors of alkaline phosphatase, L-tetramisole, and related compounds were introduced by Borgers (1973). These inhibitors make it possible to distinguish clearly between alkaline phosphatase and other phosphatases whose specificities overlap with alkaline phosphatase (Borgers and Thone, 1975 and 1976).

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