INTRODUCTION
The skin is the boundary between the organism and the environment. Its paramount function is that of protection. The skin elicits all the principal modalities of sensations, such as touch, pain, itch, tickle, warmth and cold. It prevents loss of water, electrolyte and organic substances. The microstructure of the animal skin is outlined in detail by Wilson (1928) and Kuntzel (1944). The skin has three layers viz., epidermis, dermis and subcutaneous tissue. The epidermis forms a thin but efficient barrier against the environment. Three types of cells can be distinguished viz., keratinocytes, melanocytes and Langerhans cells. The keratinocytes constitute the major part of the epidermis forming the stratum corneum of 15 to 20 cellular layers in thickness (Holbrook and Odland, 1974). The keratinocytes are attached to each other by desmosomes (Chambers and Renyi, 1925). There are subtle structural modifications of desmosomes as the basal keratinocytes differentiate through granular and stratum corneum (see Odland and Reed, 1967; Raknerud, 1975).

The dermis and epidermis are attached and the zone of attachment is commonly referred to as the epidermal-dermal junction (Briggaman and Wheeler, 1975). The basal lamina is derived from the epidermis (Hay and Revel, 1963; Briggaman et al., 1971). The basal lamina is a feltwork of filamentous glycoproteins, a component of which has been identified as
Type IV collagen (Yaoita et al., 1978). Tubular microfibrils appear to be inserted into the basal lamina and are aggregated in loosely organised bundles in the subjacent papillary dermis (Briggaman and Wheeler, 1975). They have been shown to be continuous with the microfibrils associated with elastin in dermal elastic fibres (Kobayashi, 1977).

It has been suggested by several authors that the connective tissue extracellular matrix and the basement membrane of developing organs exert an essential function in cell differentiation and morphogenesis (Grobstein, 1967; see Slavkin, 1975; see Lash and Vassan, 1977; see Hay, 1978), namely in skin (Dodson, 1967; Goetinck, 1970), vertebral cartilage (Trelstad et al., 1972; Kosher and Lash, 1975), salivary gland (see Bernfield and Banerjee, 1978), cornea (see Meier and Hay 1975) and teeth (Lesot and Ruch, 1979).

The skin has different carbohydrate moieties like glycogen, glucose, acid mucopolysaccharides etc. (Johnson and Fusaro, 1972). Investigations have indicated that the glucose content of skin never exceeds that of blood and that there is a direct correlation of skin glucose to blood glucose (Urbach and Lentz, 1945; Schragger, 1962). Skin uses glycogen as well as glucose as a source of energy (Braun-Falco, 1954). Enzymes associated with aerobic and anaerobic glycolysis in skin have been demonstrated by several
investigations in several animals as well as man (Zelickson, 1960; Rippa and Vignali, 1965; Halprin and Ohkawara, 1966a, 1966b; Mier and Cotton, 1970; Kondo and Gernat-Torsellini, 1974).

The presence of tricarboxylic acid cycle and hexose monophosphate shunt in the skin was established by Adachi and Uno (1969). Halprin and Ohkawara (1966b) demonstrated the presence of glycogen synthesizing enzymes in the skin viz., UDPG pyrophosphate, glycogen synthetase, hexokinase, phosphoglucomutase, glucose-6-phosphate dehydrogenase, phosphorylase and UDPG dehydrogenase. The presence of the enzymes like glycogen phosphorylase, amylo-1,6-glucosidase and acid - glucosidase in human skin has also been established (Leathwood and Ryman, 1971).

The skin lipids are mainly derived from two sources: the sebaceous gland and epidermis. The various classes of lipids identified in the skin include fatty acids, triglycerides, squalene, sterol esters, sterols and saturated hydrocarbons (see Lewis and Hayward, 1971). The subcutaneous layer of the skin is very rich in lipid content (Kellum, 1967). Nicolaides et al. (1955) were the first to demonstrate the active lipogenesis in human skin. Palmitic acid and stearic acid are the major fatty acids identified in the skin (Vroman et al., 1969). Fatty alcohols in human
surface lipids include alcohols with both odd and even numbers of carbon atoms (Brown et al., 1954; Hougen, 1955). Aso et al. (1975) reported the capacity of skin to synthesize prostaglandins $\text{PGE}_1$, $\text{PGE}_2$ and $\text{PGF}_{2\alpha}$.

Protein synthesis occurs in all the three layers of the skin (Fukuyama and Bernstein, 1961). DNA synthesis is predominant only in basal lamina (Fukuyama and Bernstein, 1963), while RNA synthesis is reported in all three layers of the skin (Fukuyama and Epstein, 1968). Skin has abundant fibrous proteins, collagen is the most predominant among them (Astbury, 1933). The largest domain of collagen molecule contains more than 1000 amino acids in a repeating Gly-x-y triplet sequence (Kuhn, 1984), which is involved in the triple helix formation. The general sequence that can be represented as gly-x-y, where x may be proline or at times may be some other amino acids and y may be hydroxyproline or at times may be some other amino acid (Hanning and Nordwig, 1967).

Tropocollagen molecule is the basic structural unit of the collagen molecule. In the native form it is a rigid rod shaped particle of 2800 $\text{A}^0$ long and 14 $\text{A}^0$ in diameter with a molecular weight of about 300,000 daltons as revealed by physiochemical and electron microscopic studies (Ramachandran, 1967). In the formation of collagen fibre,
the tropocollagen molecules are arranged in a characteristic fashion, each molecule being displaced one quarter of its length relative to the next molecule, producing the regular repeat of about 680 Å seen in electron micrographs of native collagen fibrils (Kuhn, 1960; Schmitt and Hodge, 1970). Regulation of collagen degradation is a significant part of the homeostasis of collagen in tissues. Collagenase is the major enzyme responsible for collagen degradation (Eisen et al., 1968).

The skin possesses a number of receptors for endogeneous molecules. The presence of receptors for steroids in the chromatin fraction of epidermis has been demonstrated (Baker et al., 1977, Slaga et al., 1977; Uzuka et al., 1978). The mouse and human keratinocytes of the epidermis possess the receptors for glucocorticoids (Epstein and Munderloh, 1981; Ponec et al., 1981; Epstein and Bonifas, 1982). Estrogen receptors were concentrated in the nuclei of the cells of the dermis and the lower dermis (Stumpf and Sar, 1976; Uzuka et al., 1980).

Androgen receptors were identified in the skin (Hamilton, 1942). The normal functioning of the skin is androgen dependent (Hay and Hodgins, 1974; Kutten and Mauvais-Jarvis, 1975; Price, 1975). The skin actively reduced testosterone to dihydrotestosterone (Wilson and
Walker, 1969; Moore et al., 1975), which binds with high affinity to the dermal receptors (Svensson and Snochowski, 1979). Receptors for vitamin D₃ have been found in the human epidermis (Feldman, et al., 1980), and rat epidermis (Stumpf et al., 1979). Dermal binding of insulin (Bar et al., 1980), thyrotropin (Cheung et al., 1978), melanocyte stimulating hormone (Lerner et al., 1979) and T₃ (Bernal et al., 1978), have been established. The dermal receptors have high affinity for T₃ than T₄ (Eberhardt et al., 1979).

The presence of α and β receptors in the human epidermis has been demonstrated (Flaxman and Harper, 1975; Yoshikawa et al., 1975). The skin has receptors for substance P (see Nilsson and Brodin, 1977), Vasoactive intestinal Peptide (Lundberg et al., 1980), enkephalin (Hartschuh et al., 1979) and bradykinin (Regoli and Barbabe, 1980).

Hormones play an important role in maintaining the structural and functional integrity of the skin. Androgens increase the process of protein synthesis in the skin (Shuster and Thody, 1974). The effect of progestogens on the skin has been under debate, as Ebling (1948) stated that progestogens do not affect the skin, while this was contradicted by Haskin et al. (1953) and Lasher et al. (1954), who claimed that the action of progestogens to be
comparable to that of testosterone. Estrogens undoubtedly depressed protein synthesis (Ebling, 1973). Cortisone has been demonstrated to suppress protein synthesis (Haskin et al., 1953), while thyroid hormones stimulate the protein synthetic activity of skin (Thody and Shuster, 1970, 1972).

The synthetic activity of the skin is enhanced by TSH (Thody and Shuster, 1972), ACTH (Thody and Shuster, 1970, 1972), MSH (Shuster and Thody, 1974) and growth hormone (Ebling et al., 1975). However, prolactin and gonadotropin although had no direct effect on the dermal synthetic activity, enhance the same through testicular and ovarian steroids (Ebling et al., 1969; Nikkari and Valavaara, 1970).

Collagen being the major protein of the skin, the effects of these hormones on collagen are important. The androgens like testosterone and dihydrotestosterone increase the production of collagen in skin (Canalis et al., 1978; Centol et al., 1988). The synthesis of collagen molecule is inhibited by estradiol and progestogens (Sauada et al., 1978 Hagberg et al., 1980), Beldekas et al., 1981). Dermal collagen production was decreased also by PTH (Canalis et al., 1977), glucagon (Canalis et al., 1977) and TSH (Brahma et al., 1980). On the other hand, prolactin (Brahma et al., 1980) and insulin stimulated collagen synthesis in the skin (Bembenek et al., 1982; Kream et al; 1985).
Insulin-like growth factor also increased the production of collagen (Guenther et al., 1982).

The metabolism of collagen is altered in different pathological conditions. In epidermolysis bullosa collagenase activity was reduced leading to increase in collagen content of epidermis (Eisen, 1969; Bauer et al., 1977). There is an increase in collagen content of skin in rheumatoid arthritis (Weiss et al., 1971; Adams et al., 1970) and in fibroblast cultures affected by periodontitis (Narayanan and Page, 1976). In Ehlers-Danlos syndrome there is a marked disorganisation of dermal collagen fibrils and increased collagen biosynthesis (Counts et al., 1981). Cutis laxa is another disorder of connective tissue in which the collagen stability is lost due to lysyl oxidase deficiency (Byers et al., 1976). Osteogenesis imperfecta is one of most common disorders of connective tissue. In this condition the ratio of type I to type III collagen is decreased in the skin (Sykes et al., 1977). Another disorder is the Marfans syndrome which is due to abnormalities in the structure or processing of collagen (Neuci and Beltrami, 1968). The skin collagen content is reduced in Marfans syndrome (Priest, et al., 1973).
In diabetes mellitus there occurs a decrease in the concentration and proportion of soluble collagen in the skin and gingiva (Ramamurthy et al., 1972, 1974; Golub et al., 1977). Experimental diabetes has been shown to decrease vascularization and wound healing in the skin (Yep et al., 1972; Prakash et al., 1973; Arquilla et al., 1976). Experimentally induced diabetes decreased synthesis of collagen accompanied by increased catabolism of collagen in the skin (Mohanam and Bose, 1981; Schneir et al., 1982; Robert et al., 1988; Schneir, et al., 1988). Decrease in content of acid soluble collagen extracted from human diabetic skin has been reported (Schnider and Kuhn, 1981). Alloxan-induced diabetes in experimental animals decreased total solubility of collagen in gingiva and skin (Ramamurthy et al., 1974; Golub et al., 1977; Behera and Patnaik, 1979).

The discovery of lysosomes as a distinct group of cytoplasmic organelles containing acid hydrolases (De Duve, 1959; Novikoff, 1961) capable of degrading many different types of macromolecules (DeDuve et al., 1955) has led to the formation of concepts implicating lysosomes in a variety of pathological conditions. Indeed, accumulation and labilisation of lysosomes often occur at sites of tissue injury (Dingle, 1961; Barland et al., 1964; Janoff and Zweifach, 1964; Coppi and Bohardi, 1968; Weismann et al., 1969). It has been established that lysosomal enzymes
possess the capacity to degrade completely the components of connective tissue such as collagen (Lazarus et al., 1968; Anderson, 1969; see Woessner, 1970, 1971), proteoglycans (Weismann and Spilberg, 1968; Mahadevan et al., 1969; see Kochar and Larson, 1977), glycoproteins (Mahadevan et al., 1969) and elastin (Janoff and Zeligs, 1968).

The lysosomal cathepsin B1 can degrade native soluble and insoluble collagen (Burleigh et al., 1974). It has been proposed that proteolysis by cathepsin B1 may be physiologically important for collagenolysis (Burleigh et al., 1974; Dingle, 1975). Colchicine (Harris and Krane, 1971) and cytochalasin B (Harris et al., 1975) were showed to stimulate collagenase. Cathepsin D and \( \beta \)-glucuronidase also play a role in collagen catabolism (Werb and Reynolds, 1974).

The relation of lysosomal enzyme and collagenase activity to changes in hormonal levels have been established. Parathyroid hormone increased collagenase activity in the bone culture (Sakamoto et al., 1975). Progesterone completely abolished collagenase and lysosomal enzyme activity (Jeffrey et al., 1971). Hydrocortisone and dexamethasone inhibit collagenase and lysosomal enzyme activity (Koob et al., 1974; Dayer et al., 1976). Collagenase was inhibited by estradiol (Ryan and Woessner, 1975).
Number of reports have indicated increased activity of acid glycohydrolases viz., $\beta$-glucuronidase, $\beta$-N-acetyl glucosaminidase and $\beta$-galactosidase in the plasma or serum of diabetic patients (Bomback et al., 1976; Kohler et al., 1979; Price and Foster, 1979; Merimee et al., 1981; Alhadeff and Holzinger, 1982; Miralles et al., 1982). Belforle et al. (1975) have suggested that the increased serum activities of lysosomal hydrolases as observed in diabetic patients may be due to decreased lysosomal stability. It has been shown that the activities of certain lysosomal enzymes viz., cathepsin B1, cathepsin D, $\beta$-glucuronidase and $\beta$-N-acetyl glucosaminidase were increased in the skin and serum of streptozotocin and alloxan induced diabetic rats, thereby indicating the possible alterations in the stability of lysosomes (Mohanam and Bose, 1983).

The lungs are paired organs, situated on either sides of the thoracic cavity. It is surrounded by a membrane called visceral pleura (see William and Warwick, 1980). The lungs are divided into distinct lobes by structures known as fissures and there are morphological differences between the right and left lung. The left lung of human is partially divided into two lobes (superior and inferior) while the human right lung is divided into three lobes (superior, middle and inferior) (see Anthony and Thibodeau, 1983). In the rhesus monkey, the left lung is divided into three lobes
and the right consists of four lobes. The fourth lobe is an addition of the azygos lobe (Accessory lobe) (see Hartman and Straus, 1933).

In mammals, the trachea is attached to the inferior aspects of the larynx. The trachea terminates by branching into two bronchi. Each primary bronchus now enters the respective lung on its side and divides immediately into smaller secondary bronchi. These secondary bronchi divide and branch to give rise to tertiary bronchi and arterioles. The bronchioles divide further into smaller tubules terminating in alveolar ducts, which terminate into several alveolar sacs, the walls of which consist of numerous alveoli (see Anthony and Thiobodeau, 1983).

The major function of the lung is purification of blood by the transfer of oxygen from the environment into blood and liberation of carbon dioxide from the blood to the environment (see Guyton, 1976). Histologically the lungs can be divided into parenchymal and nonparenchymal cells. The alveoli, alveolar ducts and capillaries are the parenchyma while the non-parenchyma includes conductive airways and blood vessels and coarse connective tissue structures (see Kuhn, 1976). The parenchymal cells of the lungs consist of four major types of cells, such as alveolar type I (4%), alveolar type II (6%), endothelial cells (34%) and mesencymal
cells (43%). (Fulmer and Crystal, 1976; Kuhn, 1976). The alveolar type I cells cover the surface of the lungs. Type II cells are metabolically active and the responsible for the elaboration of surfactant of the lung (Caulet et al., 1968). The phagocytic and secretory functions are also carried out by the type II cells.

The major cells of the non-parenchymal portion of the lung are the cells of the conductive airways which include epithelial cells (globlet, ciliated, brush, basal and glandular cell of various types), smooth muscle cells and connective tissue cells (chondroblast and mesenchyma) (see Sorokin, 1972). The ciliated cells (50%) and glandular cells (10%) are the most predominant epithelial cells (see Kilburin, 1974).

Cells containing large number of dense-cored granules are present at all levels of the tracheo-branchial tree (Bensch et al., 1965; Gmelich et al., 1967; Lauweryns and Peaskens, 1969; Culz and Conen, 1972; Tateishi, 1973) and have been recognised at the alveolar levels as neuroepithelial cells (Lauweryns et al., 1973). These cells occur individually interspread among other epithelial cells of the airways and into organised structures (Lauweryns et al., 1972, 1973; Hung et al., 1973). Similarly, the endocrine-like cells are also most predominant in the small
and large bronchi, bronchioles and the ducts of mucus glands of human lung (Tateishi, 1973).

There are specialised cells on the surface of the alveolar cells called macrophages which originate from the bone marrow stem cells (Bowden and Adamson, 1972). These cells have the capacity to absorb foreign materials by phagocytosis (see Green, 1970) and to produce collagenase (Wahl et al., 1974, 1975). The lungs synthesize more antibodies than any other organ (Askonas and Humphrey, 1958; Humphrey and Sulitzeanu, 1958). The unencapsulated lymph nodes of the hila of the lungs near large blood vessels are involved in the synthesis of antibodies (Bienenstock, 1973).

The lungs contain quite a number of lipids. The lung surfactant is mainly composed of phospholipid, dipalmitoylphosphatidylcholine (Hawthrone and Ansell, 1982). Phospholipase A2 which catalyzes the hydrolysis of ester bond in position 2 of glycerophospholipids to form a free fatty acid and lysophospholipid is present in lungs (Bell and Coleman, 1980). The lipogenic enzymes are in the active state in the lungs (see Boyer, 1983). The lungs have triacylglycerol, phosphoglycerols and sphingolipids (see Vance and Vance, 1985). Lungs contain relatively high concentration of prostaglandins (PGE2 and PGF2α) and enzymes involved in the synthesis and degradation of prostaglandins.
(Karim et al., 1968; see Mason, 1976). Lungs also contain prostaglandin-E-9-keto reductase, an enzyme which converts the prostaglandin E to F type (Lee and Levine, 1974). In general PGE series is thought to relax pulmonary bronchial muscle and F series cause contraction of the same (see Cuthbert, 1973). Vitamins are important for lung function. Cytoplasmic receptors for vitamin A were identified in rat lungs (Bashor et al., 1973). This has an important function in connective tissue synthesis (Ehrlich et al., 1973).

The major carbohydrate of the lungs is glucose. Glucose is an important metabolic substrate of lungs participating in a variety of biochemical reactions (Hers and Van Schaftingen, 1982). One fifth to one quarter of the utilized glucose is oxidized to carbon dioxide. Conversion of glucose into amino acids and incorporation into protein account for one-tenth of the utilized glucose. One such protein being collagen.

The protein composition of the lung is mainly constituted by collagen and elastin which form the framework of lung. There are at least 5 genetically distinct collagen (Called isotypes) found in normal lung (Madri and Furthmayr, 1980; Laurent, 1981). Each type of collagen has distinct physical and chemical properties. Type III collagen which is located in the interstitium of the lung parenchyma (Madri
and Furthmayr, 1980; Bateman et al., 1981) is the most abundant of these collagens.

There are several reports which reveal the fact that lungs have specific receptors for various hormones. The specific of β adrenergic receptors in the fetal lung of rats has been demonstrated (Whitsett et al., 1981). Both β₁ and β₂ adrenoreceptor are present in the human lung (Uraban and Krystyna, 1985). Of these two receptors the β₁ type receptors predominate over β₂ receptors (Philip et al., 1985).

Specific binding sites for insulin (see Walter, 1980), thyroid hormones (Marjorie et al., 1979; Das and Ganguly, 1981; Philip and Linda, 1985), progesterone, testosterone (Lyons and Antonio, 1969) see Walter, 1980; Savitas et al., 1983), corticosteroid (Ballard and Ballard, 1972) and prolactin (Amit et al., 1984) in the lungs have been established. There are about 9500 nuclear binding sites and 12000 cytoplasmic binding sites per fetal rabbit lung cell (Ballard and Ballard 1972). Hosokawa and Takeda (1980) demonstrated the presence of estrogen receptors in the lungs.

Glucocorticoids inhibited the accumulation of collagen in the lungs (Sterling et al., 1982). This was achieved by selectively inhibiting the synthetic enzyme, prolyl hydroxylase (Sterling et al., 1982). 17β-Estradiol 3-
benzoate, a synthetic estrogen increased collagen synthesis in the mouse lungs (Hosokawa and Takeda 1980).

In certain pathological conditions there occurs changes in lung collagen. In Ehlers-Danlos syndrome there occurs fragmentation of collagen fibres in the lungs (Jansen, 1955). The lungs are altered in particular in cutis laxa (Beighton, 1972) and the collagen of lung is disorganised (Goltz et al., 1965). In Marfan's syndrome there is an increase in lung collagen (Goodman and Dorney, 1969). There is a large deposition of collagen fibres in the lungs and heart and skin in alcaptonuria (O'Brien et al., 1963). Milch (1962) reported accumulation of lung collagen in ochronosis.

Collagen which forms a major part of the lung matrix is of importance for the normal functioning of lungs (Ofulve et al., 1988a). In diabetes mellitus there occurs increase in this macromolecule of the lung (Sahebjami and Denholm, 1987, 1988; Ofulve et al., 1988, Ofulve and Thurlbeck, 1988). The increase in lung collagen in diabetes is in part due to reduced breakdown of the connective tissue proteins (Ofulve and Thurlbeck, 1988).

The incorporation of $^{14}$C-lysine and $^{14}$C-hydroxyproline are elevated in diabetic glomeruli (Cohen and Khalifa, 1977). The amount of DNA and elastin were also increased in diabetic rat lung. The crosslinking of collagen
was less in these conditions in the lung (Reisner et al., 1987).

Though much has been worked out on the biochemical changes in the skin of diabetic rats there are few areas which have not been given the required importance. Though a number of reports are available on dermal collagen and few lysosomal enzymes like cathepsin B and D, \( \beta \)-glucuronidase, \( \beta \)-N-acetyl glucosaminidase in diabetic rats, not much attention has been focussed on the glycosidases in relation to collagen content. Collagen being a glycoprotein, study of glycosidases like \( \alpha, \beta \) glucosidases, galactosidases along with arylsulfatase and acid phosphatase will be a worth while attempt in correlating the concentration of collagen and its degrading enzymes.

With regard to the lungs, the present investigation itself can be considered as a pioneering effort to study the collagen in relation to the lysosomal enzymes due to diabetes as no information is available to date. Hence, the present investigation would possibly throw some light in this area of research. Further this will help to draw a comparison between the skin and lung in regard to alterations in the level of collagen in relation to the alterations in the activities of lysosomal enzymes due to diabetes mellitus.