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ANATOMY

MICROSCOPIC STUDY OF BASEMENT MEMBRANE IN OESOPHAGUS OF HUMAN BODY WITH COMPARATIVE STUDY OF LABORATORY ANIMALS

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Abstract

The basement membrane is a term, originally given to a layer of variable thickness and distinction found at the basal surfaces of epithelia Michel Ross, (1989). Since the layer was positioned immediately below the bases of the epithelial cells, this layer was termed basement membrane. Basement membrane serves as a selective filtration barrier to substances, moving between the interstitium and the parenchymal cells.

Pierce, Midgley and Ram (1964), were the first to classify the basement membrane under two structural headings: a) Homogeneous Lamella, (b) Fibrillar Lamella. Arthur Ham (1979), described the basement membrane as a thick structure less membrane just below the tracheal epithelium. It was later believed that all epithelial cells rested on such a membrane although generally less thick than that in trachea. This extracellular supporting layer has been interpreted by William Bloom and Don Fawcett (1968), as a condensation of the ground substance of the connective tissue, at its interface with the epithelium. The basement membranes are laminae of dense amorphous material, which vary in thickness and are associated with many types of cells, embedded in or adjacent to connective tissue eg. Schwan cells, capillary endothelium and epithelia in general, Kefalides N.A. (1973). In some situations the basement membrane may be particularly thick easier to investigate, as in the glomerular membrane and so, cornea and deep to the mucous lining of trachea. It is believed that the epithelia secrete a special variety of cell coat or g1ycocalyx along their basal surfaces where they border underlying connective tissue. This material consists of mucoprotein matrix within which very fine matted filaments of a special type of collagen are embedded. This mat is termed the basal lamina. Bailey (1978). A similarly disposed layer around the basal surface of kidney tubules is almost entirely composed of basal lamina. In other locations such as ureter, where beneath the transitional epithelium, the basement membrane is so thin as to be un-resolvable by light microscope. The basement membrane is often difficult to see in routine Haematoxylin and Eosin (H & E) preparation, but can clearly be demonstrated by staining with Periodic Acid Schiff (PAS) or with silver impregnation methods. Basement membrane then appears as a thin continuous layer applied to the base of the epithelium. Basement membrane is not a single structure, but has two or more distinct components that are not resolved as such, with light microscopy. However, electron microscope showed that the deeper part of the basement membrane was actually composed of a matted network of reticular fibres. The basal lamina is usually about 80 rim thick and consists of fibrillar layer lamina densa (20 to 50 nm wide) and lamina lucida, intervening between adjacent cell membrane and lamina desna. The lamina lucida shows granular or fibrillar features, regularly spaced. The lamina densa is composed chiefly of a delicate network of type IV collagen fibrils and heparan sulphate proteoglycan. Lamina lucida has been shown to contain laminin, fibronectin and various proteo81ycans. These three molecules may be important in adhesions of cells to the basal lamina and adhesion of the basal lamina to the connective tissue matrix. Outside the basal lamina are small fascicles of unit fibrils of collagen and reticular fibres, embedded in the amorphous protein polysaccharide ground substance. All these components which, include basal lamina, reticular fibres and ground substance contribute in the formation of basement membrane. The chemical composition of basement membrane is 90% protein, 8% carbohydrates and 2% lipids. There are approximately equal amounts of collagen like and non-collagen proteins, the latter being g1ycoproteins, containing glucose, galactose, mannose, hexosamine and some sialic acid. This is amply demonstrated in their staining reactions. The reticular fibres are mainly responsible for its impregnation with silver' salts and appear black. While the Periodic-Acid-Schiff reaction (PAS) involves the polysaccharides of the basal lamina and the ground substance, and appear magenta. Chemical studies of isolated basal lamina from the kidney, indicate that, their main structural component is a form of a collagen. There is now considerable evidence that this layer is a product of the overlying epithelium and not a condensation of the underlying connective tissue ground substance. However, this is still disputed [William Bloom and Don Fawcett (1968)]. The reticular fibres and their associated polysaccharide matrix are mainly the products of connective tissue fibroblasts. The basal lamina was found to be always following the contours of the basal surface of the epithelium. Basement membrane provides attachment for the epithelium to the underlying connective tissue and to influence the differentiation and proliferation of the epitheial cells that contact it. There has been much interest in the role of basement membrane in regeneration of the peripheral nerves after injury. Here basal lamina components appear to be involved in guiding outgrowths of axon and the re-establishment of fibre continuity. Besides, this knowledge is being increasingly applied to the clinical field.In diabetes there is a

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great prominence of the capillaries due to thickening and reduplication of the basement membrane, the fibrillar structure of which is markedly exaggerated. In most of these situations the individual layers of basement membrane have probably been deposited there, by now cell generations, give an indication of the number of cell generations which have occurred at that particular site, like rings on cross section of tree trunks. One of the prominent features of alveolar reaction to injury, is thickness of alveolar septum caused by expansion of interstitium leading to an increase in Air- Blood-gas exchange distance. In acute alveolar injury, in which the interstitial edema is prominent, there appears to be no separation of interstitial edema is prominent, there appears to be no separation of composite basal laminae, although the thick part of the alveolar septum is greatly expanded by edema field. In chronic alveolar injury, the interstitial reaction is dominated by fibrosis. The hyperplastic granular pneumocytes are frequently seen resting on the basal lamina facing connective tissue. The significance of these changes and their specific effect on tissue function is still unclear. Hence an attempt has been made to study the structure of the basement membrane under light microscope, in selected organs of human and laboratory animate be staining with special techniques and compare the results.

Keywords: Microscopy, Basement membrane, Oesophagus

Introduction

The structure of cells, tissues and organs can be studied in great detail with the help of microscope. Microscope dates from 17th Century when Robert Hooke and Marcelle Malphighi employed simple lenses in the study of various structural features. Between 1673 and 1716 Leuwenhock developed compound lenses. Microscopic anatomy developed slowly during the 18th century and by the early 19th century the compound microscope had become highly developed. The phase contrast microscope was devised by Zernike in 1932. The present burst of activity resulting from increased magnification and resolution is possible with the electron microscope.Bichat 1801, has been described as Father of descriptive Anatomy. He compared the minute structure of the animal body to the substance of a web or woven fabric. The word Bichat used was the old French term, “tissue”. Hence arose the name Histology (Histo-web) for this new method of studying the structure of animals. Jacob Henle (1837), first described the microscope structure of human epithelium. He was the first to describe, the epithelium of skin and intestine, to define columnar and ciliated epithelium and to point out the epithelium as a living membrane of all free surfaces of body, and Histology of kidney.William Bowman (1842), an English physiologist and ophthalmic surgeon was he first to describe the basement membrane in kidneys. Willelm His (1865), invented the microtone and using serial sections and the wax method, drew the section on wax plates, then placing them together demonstrated morphological relations in three dimension. Wilhelm His will be remembered for the science of origin of tissues (Histogenesis). He pointed out a membrane between epithelial cells and connective tissues of skin. He also described basement membrane while working on the origin of tissues. Frieboes (1920), visualized the basement membrane in skin as a complex argyrophilic reticulum. Gersh and Catchopole (1949), have described the organization of basement membrane and ground substance and their significance, in tissue injury. Bloom, Hartmann and Vernier (1959), found out the relative thickness of the basement membrane of normal kidney golmeruli in man. Kurtz and Fieldman (1962), described the development origin of the basement membrane. They stated that the basement membrane is developed from the adjoining connective tissue cells. Pierce, Midgley and Ram (1964), described the histogenesis of basement membrane and worked on its epithelial origin.William Bloom and Don Fawcett (1968), described that the basement membrane is present between epithelium and the underlying connective tissue, as an extracellular supporting layer and has been interpreted as a condensation of the ground substance of the connective tissue at its interface with the epithelium. The basement membrane is often difficult to see in routine haematoxylin and eosin preparations but can be clearly demonstrated by staining with PAS or with silver impregnation. It then appears as a thin continuous layer closely applied to the base of the epithelium. Outside the basal lamina are small fascicles of unit fibrils of collagen, (reticular, fibers), embedded in an amorphous protein polysaccharide ground substance. All these components basal lamina, reticular fibers and ground substance- contribute to the image of the basement membrane seen with the light microscope. Chemical studies of isolated basal lamina from the kidney indicate that their main structural component is a form of collagen. This layer is a product of overlying epithelium and not a condensation of the underlying connective tissue ground substance. The reticular fibres and their associated polysaccharide matrix on the other hand are mainly the products of connective tissue fibroblasts. Kefalides (1973), describes basement membrane as a lamina of amorphous material which varies in thickness and is associated with many types of cells embedded in or adjacent to connective tissue eg. Schwan cells, muscle cells and cells of capillary endothelium and epithelium. In some situation they may be particularly thick and so incidentally easier to investigate as in glomerular membrane of the kidney, the lens capsule and the anterior limiting Descmet’s membrane of the cornea. Such membranes may form supporting layers for cell attachments to restrict the passage of large molecules and to anchor the adjacent cells to connective tissue

fibres. Ultra structurally the basement membrane usually consists of two zones: a basal lamina lying close to the plasma membrane of the neighbouring cell layer and a more diffuse reticular lamina, which merges into the adjacent connective tissue matrix. The basal lamina is usually about 80 nm thick and consists of a fibrillar layer the lamina densa (20-50 nm wide) with an electronlucent zone, the lamina lucida-intervening between the adjacent cell membrane and the lamina densa. The lamina lucida shows granular or fibrillar features regularly spaced. The lamina densa is composed chiefly of a delicate network of type IV collagen fibrils and heparin sulphate-proteoglycan, whereas the lamina lucida has been shown to contain laminin, fibronectin and various proteoglycans. These latter three groups of molecules may be important in the adhesions of cells to the basal lamina and adhesion of the basal lamina to the connective tissue matrix. The reticular lamina is composed of condensed connective tissue matrix, including a reticulum of fine collagen (type III) in the form of reticulin fibres. The layer may be thick and is the main component of the total basement membrane which can be demonstrated at the light microscope level with such staining technique as PAS reaction. Rudolf Vracho (1974) described the basement membrane in his article- Basal lamina scaffold – Anatomy and significance for maintenance of orderly tissue structure. The basal lamina is an extracellular scaffold positioned between parenchymal cells and connective tissue. Parenchyma cells attach to one of its surfaces and the other surface is anchored to connective tissue. By its presence it defines the spatial relationships among similar and dissimilar types of cells and between these cells and the space occupied by supportive and connective tissue. Replacement of cells which have died during normal functioning or have become damaged in course of injury occurs with new cells in an orderly manner. This is along the framework of the basal lamina scaffold. This process appears to be aided by the polarity of the basal lamina and by an apparent specificity of cells types, and it enables multicellular organism to reconstitute histologic structures of most tissues and organs to what they were, prior to loss of cells. If the basal lamina is destroyed, the healing in most tissues results in formation of scar and loss of function. The properties of the basal lamina, concerned with maintenance of histologic order in organs and tissue offer new ways to interpret the pathogenesis of several common disorders including emphysema, scars, adhesions and cirrhosis of liver. Excessive accumulation of basal lamina material also occurs in patients with diabetes mellitus.

Rolland Lesson C. and Thomas S. Lesson have described basement membranes as sheets of extra cellular material present under the basal surfaces of epithelial cells, around muscles, nerves, capillaries, and fat cells, and situated between these elements and the underlying or surrounding connective tissue. Basal laminae vary in thickness, are rich in mucopolysaccharides and have high content of collagen,. They stain intensely with PAS and sliver techniques but are poorly demonstrated in H &E preparations. Basal laminae are synthesized by the related cells and act as diffusion barrier to rapid ion exchanges, selectively changing molecular and ionic diffusion rates. Junqueria, Corneiro and Contopoulos (1977), have described that all epithelial tissued have on their surface, in contact with connective tissue, a continuous sheet like structure called the basal lamina. This structure is visible with electron microscope as a thin granular deposit in which very fine fibrils may be observed to form a delicate network. It is known to contain an amorphous protein polysaccharide complex and the protein-collagen. Results obtained with immunofluor suggest that epithelial cells are probably the main source of basal lamina. Although the thickness of the basal lamina is variable (50-80 nm) if does not present a barrier to the diffusion of most substances. Since epithelial tissues are avascular, basal laminal permeability to substances is a pre-requisite for proper nutrition and function of the epithelia. In the epithelial tissues (eg. The skin, which are subject to friction, the basal lamina is anchored to the subjacent connective tissue, by special small fibres and collagen is inserted vertically into the connective tissue. On the basis of their function these structures are called anchoring fibres. In some tissues such as skin, kidney, glomerulus and renal tubules, fibrils of collagen (reticular fibres) embedded in an amorphous protein polysaccharide constitue another layer of the basal lamina. This considerably thicker structure is clearly visible with the light microscope when stained by two methods. The reticular fibres can be impregnated with silver salts and the amorphous component has a strong PAS positive reaction. All of these constituents- basal lamina, ground substance and reticular fibres-form what is called the basement membrane. Bailey (1978) described the basement membrane beneath most epithelia and that it is the combination of basal lamina and reticular lamina, which is visible by light microscope. On the other hand similarly disposed layer around the basal surfaces of kidney tubules is almost entirely composed of basal lamina only. The basal lamina appears as a thin deposit very early in the development of most epithelial rudiments of embryo. It seems to serve an important function in the segregation of tissues within the embryo. It becomes the peripheral boundary of broad and complex connective tissue compartment. However it should not be considered an impenetrable boundary. Many molecules easily diffuse across basal laminae to interact with epithelial cells on one side and connective tissue components on the other. During development

of the embryo, as the epithelial rudiments enlarge, additional reinforcement and stabiliazation are required to maintain the different shapes of epithelia. The acquisition of basal lamina around epithelial rudiments may be the first step in the extra cellular stabilization.

Eventually collagenous and reticular fibres may also be laid down mostly through connective tissue cells, reaching reticular laminal proportions. Thus an extracellular collagenous glycosaminoglycan encasement serves as a reinforcing frame work.

Epithelia are not the only tissue which deposit basal lamina material. During embryonic development certain other cells segregate themselves from surrounding connective tissues by the acquisition of similar coats. When the coat completely or nearly completely surrounds such cells as in the case with smooth, skeletal, and cardiac muscle, as well as fat cells, the encasement is more properly termed as external lamina. Reticular lamina is seldom well developed in association with external lamina.

Arthur Ham (1979), described basement membrane as a structure less, special layer and it was noticed between an epithelial membrane and the connective tissue, immediately beneath it. Since it was positioned immediately below the bases of ht epithelial cells, this layer was termed basement membrane. Basement membrane was first thought to consist of homogenous condensed layer of intercellular substance produced by fibroblasts of the underlying connective tissue and was PAS positive. Basement membrane was thought to be composed of a single layer. However, electron microscope showed that the deeper part was actually composed of matted network of recticular fibres. By means of immuno-fluorescence technique for locating specific proteins, a unique form of collagen in lamina, the staining PAS positive has been demonstrated. R. Timpl (1982), described the structure and metabolism of basement membrane. Basement membranes are extracellular matrices with a characteristic morphological appearance and are composed of unique glycoproteins and proteoglycans. The major constitutents of this matrix are type IV collagen and laminin. J.T. Ireland (1982), described the renal structure with diabetic lesion. The glomerular filter is composed of three layers 1) Capillary endothelium, 2) Basement membrane and (3) Epithelial cells (Podocytes). The endothelium has large slit like pores, between 50 to 100 nm in diameter, which are much larger than the macromolecules on the plasma, so that the endothelium is no anatomical barrier to filtration. Human basement membrane is 250 to 350 nm thick and is composed of collagenous and non-collagenous material. It is rich in carbohydrates and contains glycosminoglycans which make it strongly anionic.

Both hyperglycaemia and lack of insul in experimentally increase the enzymes involved in basement membrane thickening. It may take 20 years for the membrane to reach the double the normal thickness. Non enzymatic glycosylation may further increase the thickening in hyper glycaemia. Simultaneous with basement membrane thickening there is loss of anionic sites leading to increased permeability, first seen as micro and later as macroalbuminuria. Once basement membrane thickening is established fibrin deposition within the more permeable membrane is evident on electron microscopy and ultimately plays a significant part in the formation of diffuse and nodular glomerulosclerosis. Both basement membrane thickening and mesangial englargment obstruct renal plasma flow. Hypertension by increasing int raglomerular pressure may further accelerate fibrin deposition within the basement membrane.

Micheal Ross, Edward Reith, Romrell (1989), describe basement membrane as a term originally given to a layer of variable thickness and distinction found at the basal surface of epithells. It was believed that this layer consisted of a condensed gel like substance and delicate reticular fibres which together seemed to attach the epithelium to the underlying connective tissue. In most organs the basement membrane appears much thinner and is usually indistinct but basement membrane is thick in trachea.

Basement membrane gives positive reaction with PAS, appearing as a well defined pink layer. Basement membrane includes not only the basal limina, but also a secondary layer the reticular lamina which is comprised of small unit fibrils of collagen. The reticular lamina which is comprised tissues and it is not a product of epithelium. Reticular lamina reacts with silver stain while, PAS stains principally the polysaccharides of the basal lamina. Rainer, Gotz and Thies (1990) have studied appearances of laminin, heparin sulphate proteglycan and collagen type IV during intial stages of vascularization of the neuroepithelium of the mouse embryo. The appearance of basement membrane components laminin, helparan sulphate proteoglycan and type IV collagen was investigated by indirect immune-flurorescence during 10^{th}, 11^{th} and 12^{th} days of development. All three basement membrane components could initially be seen in the embryonic C.N.S alage, along with the appearance of capillaries in the neuroepithelial cell layer. From the direct correlation between vascularization and appearance of basement membrane components in the embryonic analge one can assume that the basement membrane components laminin. Heparan sulphate, protoglycan and collagen type IV are involved in the intial vascularization of C.N.S. analge of 10-12 days old mouse embryos.

Hijikata and Sakai (1991) have studied the basement membrane in rat proximal tubule. The basement membrane of the proximal tubule segment is

thick and possesses several structural specializations that are rare in other basement membranes. These include intraepithelial ridges, dense bars, and basement membrane vesicles. The specialization are best developed on the concave side of the tubular bends were the circumferential stresses caused by the intraluminal hydraulic pressure are presumably the largest.

Material and Methods

The present study was conducted on basement membrane of oesophagus and kidneys. The tissues were obtained from laboratory animals viz. Rat, Rabbit, guines pig and Hamster and from human dead body. The tissues were removed within 6 hours after death and were cut into small pieces measuring 0.5 cms to 1.5 cms thick and immediately put in 10 % formalin in a wide mouthed bottle using separate bottle for each animal. Tissues were allowed to fix for 24 hours. Taking tissues of one animal at a time, the tissues were washed in tap water, dehydrated with 70% alcohol for 8 hours, 90% alcohol for 16 hours and absolute alcohol for 8 hours. The tissues were later cleared in chloroform and embedded in molten paraffin wax at 60^0 C. a solid block was obtained by filling a mould of suitable size with molten paraffin wax. Sections of 5 microns were taken using Spencer's rotary microtone.

Staining: The various staining techniques used for the study of Basement membrane were: Haematoxylin and Eosin, Van Geison's stain, Periodic-Acid-Schiff, Silver nitrate stain.

Staining Techniques: Haematoxylin and Eosin Stain-Haematoxylin is the most widely used and versatile dye in histological technique and is used in stains for the demonstration of cell nulei, however, elastic fibres, fibrin, neurologists and muscle striations is most commonly used as a nuclear stain preceding staining of cytoplasm and connective tissue with acid dyes like eosin.

Procedure: The sections were treated with xylol to remove wax. The xylol was then removed by treating the sections with absolute alcohol. The section was then passed through descending grades of alcohol and distilled water for hydration. The section was stained with haematoxylin for eight minutes and later washed in running tap water for 2-5 minutes till the sections blue.

The section was examined under the microscope at this stage to confirm the degree of staining. If the staining was insufficient it was stained again. If staining was in excess, it was removed by decolouring in 1% hydrochloric acid in 70% alcohol for a few seconds. Once the blue colour was regained, decolourization was stopped by washing it in alkaline running tap-water. The degree of staining was again checked microscopically till the appropriate results were obtained. The slide was then stained with eosin for 3 minutes, dehydrated in alcohol, cleared in xylene, mounted in D.P.X. and examined under the microscope. Results: Nuclei-Blue; Cytoplasm-Pink

2) Van Geisons Stain: Van Geison's staining technique is the simplest method for the differential staining of collagen. Its main disadvantages are its inability to stain young fibrils, while staining mature collagen fibres deep red.

Procedure: The sections were deparaffinized in xylol. The xyll was removed by treating the section with absolute alcohol. The section was then passed through descending grades of alcohol and distilled water for hydration.The sections were stained with Weigert's haematoxylin for 10 minutes, washed in distilled water. They were further stained with Van Geisions solution for 3 minutes. The sections were dehydrated with picric alcohol, cleared in xylene mounted with D.P.X. examined under the microscope. Results: Nuclei - Brown black to black; Collagen- Deep red; Muscle Cytoplasm- Yellow.

Periodic Acid Schiff Reaction: PAS reaction is a truely a histochemical method and is a development of Feulgen reaction for the demonstration of deoxyribonucleic acid. In the Feulgen reaction hydrolysis with hydrochloric acid liberates aldehydes and these recolour Schiffs reagent. The PAS reaction will demonstrate the sites of glycogen, staining it magenta.

Solutions:

1) 1% Periodic Acid: 1% periodic acid prepared by mixing 1 gram of periodic acid and 100 ml of distilled water was kept as stock solution.
2) Schiffs Reagent: This was prepared for fresh use as follows: 1 gram of basic fuchsin was dissolved in 200 ml of hot distilled water and brought to boiling point. After cooling to 60^0C it was filtered. 20 ml of 1% HCL and 1 gram of anhydrous sodium bisulphate was then added. The solution was further kept in dark for 48 hours. 2 gram of charcoal was added and filtered to obtain a straw colored filtrate, which was stored in refrigerator.

Procedure: The sections were deparaffinized in xylol. The xylol was removed by treating the section with absolute alcohol. The section was then passed through descending grades of alcohol and distilled water for hydration.

The sections were oxidized for five minutes in 1% periodic acid. Later they were washed in running tap water for five minutes and rinsed in distilled water. The sections were then stained with Schiff's regent for 15 minutes and washed in running running tap water for 5 to 10 minutes until the magnate color developed. If over stained, decolonization with 1% acid alcohol was done. Further the sections were counter stained haematoxylin for 1 minute and washed in running tap water for five minutes. After this the sections were dehydrate in absolute alcohol, cleared in xylol and mounted with D.P.X. Results: Nuclei- Blue: PAS positive substance- Magenta.

Silver Nitrate Stain (Nasser and Shanklin 1962) Recticular connective tissue or reticulin, consists of time branching fibres which give a supporting frame work to the rechly cellular tissues of the lymphoreticular system and to other solid organs. They are largely invisible in haematoxylin and eosin stained sctions. They are argyrophilic and silver impregnation methods are necessary for their complete domonstration.

Solutions

1) Acidified Permanganate Solution: Equal quantity of 0.5 percent potassium permanganate and 0.5 percent sulphuric acid were used, This solution was made at the time of use only.

2) 2% oxalic acid.

3) Silver Pyridine Solution : 2% silver nitrate (A.R.) prepared. At the time of use 73 drops of pyridine to every 10 ml of silver solution were added.

4) Silver Solution : To 2 ml of 0.880 Ammonia in a flask 14 ml of 10% silver nitrate solution was added carefully, drop by drop, constantly shaking the flask until a faint opalescence persisted. This was then diluted with equal volume of distilled water.

5) Reducing Fluid : Equal parts of 2 ml, of neutral formalin with equal parts of absolute alcohol.

6) 5% Sodium thiosulphate.

Procedure: The sections were deparaffinized in xylol. The xylol was removed by treating the section with absolute alcohol. The section was then passed through descending grades of alcohol and distilled water for hydration. The sections were oxidized in Acidified permanganate solution for 1-2 minutes until section become brown. Then rinsed with distilled water and decolurized with 2% oxalic acid for 1-2 minutes and later rinsed in 95% alcohol. The sections were then treated with silver pyridine solution for 50minutes at 50°C in the incubator. The sections were rinsed quickly in 95% alcohol and impregnated in Ammonical silver solution at 50°C for five minutes. Again rinsed quickly in 95% alcohol and treated with reducing fluid for two minutes. Sections were dehydrated with absolute alcohol then cleared in xylol and mounted in D.P.X.

Results: Reticulin fibres -- black; Collagen fibres -- Golden brown. 1) The section was not toned, 2) Nuclei did not stain, 3) No granular deposit within the cells.

Results

Human oesophagus

1) Haemato xylin & Eosin : The sections of oesophagus show the classical layers of mucosa, submucosa, muscularis externa and adventitia from within outwards.

The mucosa consists of stratified squamous nonkeratinized epithelium, the basal layer of which is resting on basement membrane. The basement membrane separates the epithelial layer from the lamina propria, The nuclei of the cells were stained blue and the cytoplasm pink. The lamina propria showed various fibres stained pink-mainly the collagen fibres - and cells like fibroblast, macrophages, neutrophils, lymphocytes and some capillaries containing R.B.CS. Muscularis mucosa was seen next to lamina propria and was stained pink and the nuclei blue. The submucosa showed connective tissue. The rnascularis externa consisted of two layers-inner circular and outer longitudinal.

Basement membranes: was seen as thin pink layer at the base of the epithelial layer.

PAS: Carbohydrate moiety of the intercellular substance was stained magenta. The nuceli of the epithelial cells were stained blue and the cytoplasm pink. The basement membrane appeared thin, magenta coloured and was well defined.

Van Geison's Stain: The nuclei of the epithelium were stained black and cytoplasm yellow. The collagen fibres in all the layers were stained red. Basement membrane : Appeared as a thin red layer beneath the basal layer of epithelium. Collagen fibres were stained red.

Silver Nitrate: The background was stained from grey to light brown. The collagen fibres were stained golden brown and reticular fibres black. Basement membrane appeared as a thin fibriller black layer beneath the basal layer of epithelium.

Hamster

H & E: Epithelium was stratified squamous and was keratinized. The nuclei were stained blue and the cytoplasm stained pink. Basement membrane : At the base of the epithelial layer the basement membrane was seen as a thin pink layer. The basement membrane was seen clearly at some places and it was stained pink.

P.A.S.: The nuclei were stained blue and cytopasm was stained magenta. Basement Membrane : The carbohydrate moiety of mucopolysaccharides was stained magenta colour.

Silver Nitrate Stain: The nuclei of the epithelium were not stained. The cytoplasm was stained brown. The whole background was stained brown.
Basement Membrane : Collagen fibres were stained brown and reticular fibres were stained black.

Van Geison's Stain : The whole background was stained yellow. The nuclei were stained black and the cytoplasm was stained yellow. Basement Membrane : The collagen fibres in the basement membrane were stained red. The reticular fibres were not stained.

Rat

H & E: Epithelium was stratified squamous type and was keratinized. Epithelium consisted of well defined cellular zone. These cells were in close contact with the basement membrane. Basement Membrane: Basement membrane appeared as a thin layer and was stained pink in colour.

PAS: The nuclei were stained blue and the cytoplasm was stained magenta. Basement membrane : The carbohydrate moiety of mucopolysaccharides was stained magenta colour.

Silver Nitrate Stain: The nuclei in the epithelium were not stained. The cytoplasm was stained brown. Basement Membrane: It appeared as a thin black layer beneath the basal layer of epithelium. Reticulin fibres were stained black.

Rabbit

H & E: Epithelium was non-keratinized stratified squamous type. Basement membrane : This was seen as a thin pink layer at the base of the epithelial year.

PAS: The nuclei of the epithelial cells were stained blue and the cytoplasm pink. Basement Membrane : The carbohydrate moiety of muco polysaccharides was stained magenta.

Silver Nitrate Stain: The nuclei did not. take up the silver stain. The background was brown in colour. The epithelial cells were not clearly seen. Basement Membrane : There were fine black reticular fibres and the collagen fibres were stained brown.

Van Geison's Stain: The nuclei of the epithelium were stained black and the cytoplasm was stained yellow. Basement Membrane: The collagen fibres in the basement membrane were stained red. The reticular fibres were not stained

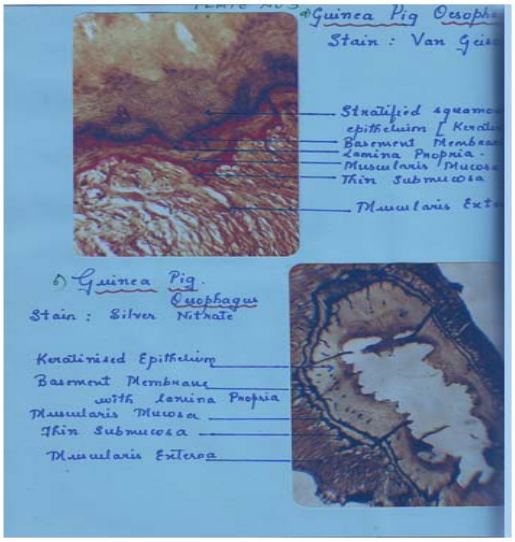
Guinea Pig

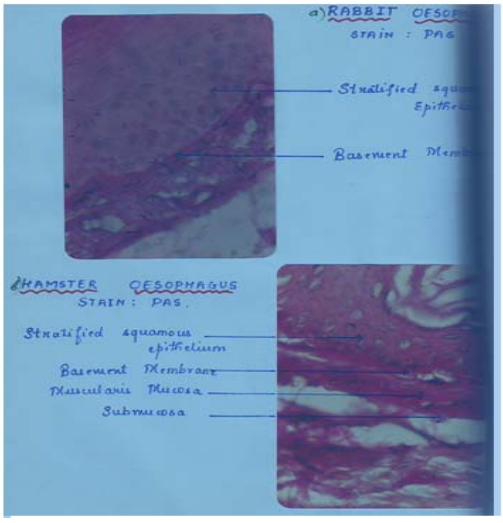
H & E: The epithelium was keratinised. The nuclei were stained blue and the cytoplasm pink. Basement Membrane: It was seen as a thin pink layer at the base of the epithelial layer.

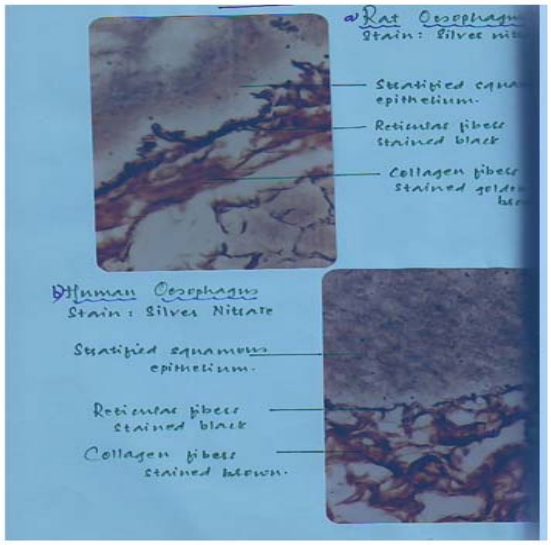
PAS: The nuclei of the epithelium were stained blue. The cytoplasm was stained magenta. Basement Membrane : The carbohydrate moiety of the mucopolysaccharide was stained magenta.

Silver Nitrate Stain: The nuclei did not take up the silver stain. The background was stained brown. Basement Membrane: Fine black reticular fibres were seen and the collagen fibres were stained brown.

Van Geison's Stain: The nuclei of the epithelium were stained black. The cytoplasm was stained yellow. Basement Membrane : The collagen fibres in the basement membrane were stained red. The reticular fibres were not stained,







Discussion

Electron microscopy has helped tremendously in the study of basement membrane. The chemical composition of basement membrane is 90% protein, 8% carbohydrates and 2% lipids. But there will be change in chemical composition of basement membrane in pathological conditions. This point suggests that the basement membrane is a complex histochemical structure. The present study of basement membrane has been done under light microscope. The basement membrane is seen just below the basal layer of the epithelium and follows the contours of the epithelium of different organs. William Bloom & Don Fawcett (1968) described that the basement membrane is present between the epithelium and the underlying connective tissue. The observations of the present study are in accordance with the opinion of Rudolf Vracko (1974) who states that the basement membrane is not seen between the cells of the same type. According to William Bloom and Don Fawcett (1968), the basement membrane is often difficult to be seen with haematoxylin and eosin stain, but can be clearly demonstrated by staining with PAS or with silver impregnation. Here it appears as a thin continuous layer closely applied to the base of the epithelium. In the present study the basement membrane was seen as a thin pink layer after staining with H & E. However, it was more clearly demonstrated after staining with PAS and after impregnation with silver salts. Frieboes (1920), visualized the basement membrane in skin as a complex argyrophilic reticulum. The argyrophilic nature of the components of basement membrane was amply - demonstrated in the present study, after staining with silver salts. Arthur Ham (1961), was also of the opinion, in the beginning that, the basement membrane appeared as a thick structure less membrane and it was thought to be a single layer. But later it was found that the deeper part was actually composed of matted network of reticular fibres and the observations of the present study resemble these findings.

Kefalides (1973), describes the basement membrane as an amorphous material which varies in thickness. The basement membrane consists of two zones, a basal lamina lying close to the plasma membrane of the neighbouring cell layer and a more diffuse reticular lamina - which merges into the adjacent connective tissue matrix. In the present study also the basement membrane was found to have two zones a basal lamina and a reticular lamina.

Bailey (1978), described the basement membrane as a PAS positive layer and that it was seen easily after H and E staining. Contrary to this in the present study, the basement membrane was not clearly appreciated after staining with H & E but it was in fact more clearly seen after staining with PAS. Arthur Ham (1979), described that the basal lamina was found to contain two different glycoproteins, one of a high and one of a low molecular weight. The presence of these glycoproteins could account for the basal laminae being PAS positive, and because the reticular fibres beneath the basal lamina would also be PAS positive it could be expected that the entire basement membrane might appear as a single PAS positive membrane. Kefalides (1973), described that the reticular lamina was composed of a condensed connective tissue matrix, including a reticulum of fine collagen fibres, known as reticulin fibres. This reticular lamina may be quite thick and it is the main component of the total basement membrane which can be demonstrated after impregnation with silver salts under the light microscope. The observations of the present study concur with the above findings.

Oesophagus: Collagen bundles appear thinner in the basement membrane of Oesophaugs of hamster when compared with the oesophagus of those of other animals when stained by Van Geison's stain. The epithelium was nonkeratinized in human and rabbit. The epithelium was thinly keratinized in hamster, rat and thickly keratinized in guinea pig. The submucosa was thick in humans and thin in all other animals No oesophageal glands were found in any of the section examined. The differential staining protocol employed in the present study was aimed at demonstrating the various components of the basement membrane.

The observations of the present study indicate that the constituents of the basement membrane may be loosely categorised as: Fibrillar components comprising of collagen bundles and reticulin fibres and Amorphous ground substance. The staining pattern observed for each of the organs in humans and animals included in the present stud demonstrates that the two constituents of the basement membrane exhibit

similar pattern of staining. The observations made in the present study indicate that the two constituents of the basement membrane do not appear to be stained with H & E to any appreciable degree. The amorphous mucopolysaccharides, stains magenta by reacting with PAS. This reaction involving the conversion of the carbohydrate moiety of the mucopolysaccharides into an aldehyde, which upon oxidation specifically stains magenta. An attempt was made to illustrate the fibrillar components of the basement membrane by demonstrating their staining characteristics by staining with Van Geison's stain and by impregnating them with salts of silver. The collagen bundles of the basement membrane were found to be stained exclusively red when the sections were treated with Van Geison's Stain. When the fibrillar components were impregnated with salts of silver, the reticulin fibres appeared distinctly black in contrast to the golden brown hue of collagen bundles, providing an interesting avenue of distinguishing the two fibrillar components of the basement membrane.

Difference in the staining pattern of the fibrillar components of the basement membrane may be due to their differential argyrophilic nature. This feature permits the delineation of fibrillar components and to elucidate the pattern of their distribution within the amorphous round substance. The observations of the present study demonstrate that the reticular fibres were found to be preferentially oriented towards the connective tissue elements. The disposition of the collagen bundles was towards the epithelial elements. This predisposition, of the fibrillar components of the basement membrane within the amorphous round substance enables them to play anchor to both the epithelial and the connective tissue elements. The precise disposition of the fibrillar components within the basement membrane may be elucidated by employing more advanced techniques. The use of electron microscopy has brought forth the intricate arrangement of these fibrillar components within the amorphous ground substance. This has enormously expanded the understanding of the basal lamina scaffold.

An understanding of the complex interplay between the two components of the basement membrane viz., the fibrillar component and the amorphous ground substance, and between these and the adjacent, epithelial and connective tissue elements, in health and disease, may unravel the nature of derangement seen in a wide spectrum of diseases afflicting the basal lamina scaffold. Vincent M. *et al* (1992.), have investigated into the pathogenesis of diabetic nephropathy and have proposed that the damage to the extracellular matrix and other tissue proteins in diabetes is caused by Maillard reaction mediated end products of advanced Glycosylation. Although several such Advanced Glycosylation End Products (AGEPSs) have been recognized, One such product pentosidine, on the collagen has been implicated in the damage caused to the extra cellular matrix of the diabetic kidney. These and other investigations of the biochemical abnormalities in the basal lamina scaffold offer interesting avenues in elucidating the precise pathogensis of the complications in the chronic diseases viz. Diabetic neuropathy, nephropathy and microangiopathy and their possible prevention.

Conclusion

An attempt was made to demonstrate the various components of the basement membrane by employing a differential staining protocol. The basement membranes of the human oesophagus and kidney were considered for the study. The basement membranes of these same organs were also studied in the animals viz. Rat, Rabbit, Hamster and Guinea Pig. The sections obtained were studied microscopically after, staining them with ; i) Haematoxylin and Eosin, ii) P .A.S., iii) Van Geison's Stain.

iv) Silver Salts.

The staining pattern observed indicated that, the staining of the basement membrane was not very distinct with H & E. While the amorphous ground substance stained magenta with P.A.S. preferentially the Collagen fibres of the basement membrane stained exclusively red with Van Geison's Stain. Impregnation of the fibrilar components with salts of silver stained reticulin fibres distinctly black while the colagen fibres were stained golden brown. This differential staining of the fibrillar components may be employed to study the disposition of the fibrillar components within the basement membrane. In the present study although the basement membrane appears to vary from organ to organ and amongst animals and human, these variations could not be discerned very clearly under the light microscope. However, the study of the basement membrane in these organs, in animals and humans may reveal more differences with the application of advanced technique of histological study.

References

Arthur Ham and David Cormack Loose Connective Tissue Text book of Histology, 8th edition J.B. Lippin Cott. 1979, 222-224.

Bichat cited in - A short history of medicine. Singer and Underwood, Oxford, 2nd Edition, 1962: 320.

Bloom Hartman and Vernier, E.M Evaluation of width or normal glomerular basement membrane in man at various ages. Anatomical records, 1959. 133, 251.

Bloom William and Don Fawcett ; Text book of histology, W.B.Saunders, 9th edition 1968. 88-91.

Bowmann William; - cited in - A short History of Medicine. Singer and Underwood, Oxford, 2nd

edition 1962. 301

Boyd William, Text book of pathology, Lea and Febiser, 8th edition, 625.

Copenhaver, Kelly, Wood Baileys Text book of Histology, 17th edition: Igaku Shoin-1978. 126-128.

Claydon E.C, Practical section cutting and staining. Nasser and Shank in method for Reticulin fibres. J.A. Churchill, London, 4th edition 112-113.

Drury & Walling : Carletons Histological Technique. General staining procedures, 125 Oxford Medical Publication, 5th edition, 1980.

Frieboes: Structure and function of skin. Academic Press, New York, 2nd edition 1962.

Gersh, Catchpole. Organisation of ground substance and basement membrane. The American Journal of Anatomy, 1949. 85, 457.

Henle Jacob ; cited in -A short History of Medicine. Singer and Underwood, Oxford, 2nd edition 1962. 322.

Ireland J.T. ; Renal structure and diabetes in Diabetes and its late complications. Eds Cudworth A.G.; Squadrito G, Anffreani D, Bodansky H, John Libbey; 1987. 5-7.

Junqueira, Carneiro & Contopoulos ; Basic Histology Section Epithelial tissue : Lange Medical Publication, 2rid edition, 1977 57-58.

Jackson A.E., R.C. Atkin, E.F. Glascow. Electron Microscopic methods of study of human glomerular cells and basement membrane in tissue culture. Anat Journal 1985. 142, 224-225.

Kefalides N.A. : Structure and Biosynthesis of Basement Membranes in - Hall D.A., Jackson D.S. (eds), International Review of Connective tissue research- cited in-Introduction, The connective tissue; Gray's Anatomy, Eds Williams P.L.,Warwick R, Dyson M, Bannister H; Churchil Livingstone, 37th edition 1989. 58-70.

Leeson and Leeson ; Histology. W.B. Saunders, 3rd Asain edition 1976. 87.

Micheal Ross, Edward Reith, Lynn Romrell ; Basement membrane, Histology text book and atlas. Willams Wilkim, 2nd edition 1989 55.

Mignot Godeau Kemency and Rebort; Basement membrane lamins collagen in control of permeability of blood brain barreir. J. Anat 1981, 133, 680.

Peirce Sri Ram Basement membrane; Am. J. Path 1964. 45(1) 474.

Rainer Herken, werner Gotz and Micheal Thies. Appearance of Laminin, herapan sulphate proteglycan and collegan- Type IV during initial stages of vascularization of the neuro-epithelium of the embryo. J. Anat 1990 169, 189-195.

Rodewald R. Karnovsky M.J Porous structure of glomerular slit disphargm in disphargm cited in Splanchnology The urinary organs' Grey's Anatomy Eds, William PL, warwick R, Dysom M., Bannister L.H,: Churchil Livingstone 37th editing 1989, 3197, 1416.

Rudolf Vracko : Basala lamina scaffold Anatomy and significance of maintenance of orderly tissue structure Am. J. Path 1974, 77, 314-346.

Sukait Hajikata, T; Structural heterogencity of the basement membrane in the rat proximal tubbule Dept. of Anatomy faculty of Medicine University of Tokyo, Japan S.O Cell tissue Res 1991 Oct. 266(1), 11-12 Document 6 of 25.

Timpla R. Structure and metabolism of basement membrane in Diabetes and its late complications Eds. Cudworth A.G Squardrito G. Andreani F. Bodansky H.; John Libbey 1987, 5-7

Thomas W. Hung composite epithelial and endothelial basal lamina in Human Lungs. The Am. J. Path, 1978, 93, 681-692

Vincent M.M Et al : Maillard reaction mediated molecular damage to extracellular matrix and other tissue protein in diabetes, Ageing & Uraemia, diabetes 1992; 41(suppl2) 34-41

Watson R.J.B.P Eyden, ultra structure observation basement membrane of human mammary gland. Anat J. 1986, 149, 239.