" INTRODUCTION AND REVIEW OF LITERATURE "

The juxtaglomerular apparatus was first recognized by Golgi (1889). This apparatus is located at the hilus of the glomerulus and consists of tubular and vascular components. The tubular component is that portion of the distal convoluted tubule which is in contact with the vascular pole of the glomerulus. This component has been termad the macula densa (Zimmermann, 1933) because at the site of contact, the nuclei of tubular cells are closely packed together than those of the contiguous distal tubule. The vascular component, on the other hand, includes the afferent and efferent arterioles,and group of cells between the arterioles (Barajas and Latta , 1967 and Barajas, 1970) . These cells have been referred to as the polkissen cell group of Goormaghtigh (Zimmermann, 1933) or the Lacis (Oberling and Hatt , 1960). Many years later, Barajas (1970) termed them the extraglomerular mesangium or the extraglomerular mesangial region because of their location between the two glomerular arterioles and because of their cytological similarity and anatomical continuity with the glomerular mesangium. The above cited definition corresponds to the mammalian juxtaglomerular apparatus . However, comparative studies of the juxtaglomerular

apparatuses have been reviewed by Sokabe (1974) , and Sokabe and Ogawa (1974) .

The terms afibrillar, epithelioid, myoepithelial or myoepithlioid cells have been used by light microscopists to designate some cells in the vascular component with clear cytoplasm, which may or may not have granules. Barajas (1979), in his research, preferred the name " juxtaglomerular granular cells " for those cells containing renin granules and the general term " agranular cells " for those cells devoid of granules.

The granular cells were first observed in the wall of the afferent arteriole by Ruyter(1925) who proposed that the juxtaglomerular granular cells were modified smooth muscle cells. Goormaghtigh(1939) postulated that they were the source of the hormone renin. Despite the presence of the granular cells in the wall of the afferent arteriole of many species, they are not observed in light microscopic preperations stained by haematoxylin and eosin. In addition, Bohle (1954) and Barajas and (1963) Latta reported their presence in the wall of the efferent arteriole.

Further investigations have fully confirmed Ryter's hypothesis. Attachment bodies, myofilaments, and structures characteristic of smooth muscle cells have been demonstrated in the cytoplasm of juxtaglomerular cells by electron microscopy. The presence of membrane-bound cytoplasmic granules appearing to originate in the Golgi apparatus (Hartroft and Neumark, 1961 ; and Latta and Maunabach, 1962) , and a well-developed endoplasmic reticulum are consistent with endocrine function. In addition, renin has been localised in the granules of juxtaglomerular granular cells by immunofluorescence (Edelman and Hartroft, 1961 ; and Hartroft and Hartroft, 1961) and has been extracted directly from these cells (Cook, 19 71) .

In most species, the granules are irregular in shape and size, usually multilobulated ,and often occupy most of the cytoplasm . In addition , the Golgi apparatus of the juxtaglomerular granular cells is well developed and may contain small granules with a crystalline structure in its cisternae(Barajas and Latta, 1965 ; Barajas,1966 ; and Biava and West, 1966) . These Golgi granules, which have been called protogranules (Barajas and Latta, 1965 ; and Barajas, 1966) are thought to be the precursors of the large granules . Protogranules are more abundant in kidneys with increased renin production, such as those with constricted renal arteries (Barajas and Latta, 1965; and

Barajas, 1966) and in juxtaglomerular cell tumours (Mac Callum, Conn and Baker, 1973 ; and Barajas,Bennett Conner and Lindstrom, 1977) than in normal kidneys. The protogranules have either rhomboid profiles with sharp angles in some species, such as man and monkey, or are oval in shape in other species such as rat(Barajas , 1966) . Other granular forms are also present in the juxtaglomerular granular cells e.g., sacs containing several protogranules observed near Golgi region, and conglomerates, where the protogranules retain their individual cryslalline pattern , These latter types have been interpreted as representing intermediate forms between the protogranules in the Golgi region and the large amorphous " mature granules " in the cytoplasm, and in some occasions contain areas of periodicity(Barajas and Latta, 1965 ; and Barajas, 1966).

As mentioned previously, the juxtaglomerular granular cells are cytologically distinctive in that they also contain myofibrils and attachment bodies. The quantity of these structures varies with the location of the cell and thus, they are more abundant in the juxtaglomerular granular cells of the afferent arteriole in the more distal portion of the glomerulus i.e., near the urinary pole,In some instance profiles of juxtaglomerular cells that contain no granules are indistinguishable from the neighbouring smooth muscle cells . Serial sections ,

however, reveal the characteristic granules at some other level (Barajas , 1970) .

The controversial results have provoked a question mark concerning the relation of the granules to renin secretion. There is no definite evidence for the exocytotic release of granules. In many studies, areas of increased density have been observed extracellular near the juxtaglomerular cells after adrenalectomy (Peter, 1976) and renal nerve stimulation (Taugner, Forssmann, Billich, Boll, Ganten and Seller,1978). These findings have been interpreted as suggestive of exocytosis . A similar interpretation have been given to the observation made by Freeze-fracturing experiments (Taugner et al.,1978) .

Casual observation of the vascular component of the juxtaglomerular apparatus reveals that many cells of this component do not contain granules. The morphologic characteristics of these " agranular cells " vary with their location ; viz, in the afferent arteriole , they are indistinguishable from typical arteriolar smooth muscle cells, whereas in the extraglomerular mesangial region , the characteristic features of these cells are scantiness of cytoplasm and

thin interlacing processes that are separated by basement membrane material. Thus, the name " Lacis " was given by Oberling and Hatt(1960). The " agranular cells " correspond to the cells of the polkissen of Zimmermann(1933) and to the pseudomeissnerian cell of Goormaghtigh(1936). The term extraglomerular mesangial cells appears most appropriate because they are morphologically similar to the intraglomerular mesangial cells and are located in a region of the juxtaglomerular apparatus that is continuous with the intraglomerular mesangium (Barajas,1979).

It is important to note that the juxtaglomerular granular cells are also present in the extraglomerular mesangium and that they increase in number under certain experimental conditions such as adrenalectomy and renal artery constriction. Granular cells could also be observed under those conditions in the intraglomerular mesangium (Goormaghtigh and Grimson, 1939 ; Dunihue and Boldosser, 1963 ; and Barajas, wang, Bennett and Wilburn , 1976) . In renal artery constriction, they were observed antero-irferiorly to the glomerulus relatively close to the hilus. It was not certain if the increase in granular cells represented an increase in the size and granularity of the already existing extraglomerular mesangial granular cells with the extension of their granulated processes

into the glomerular tuft, or otherwise due to the development of renin granules in the previously agranular extra and / or intraglomerular mesangial cells as has been reported by Dunihue and Boldosser (1963) on massive transformation of intraglomerular ones in the chronically adrenalectomised cat .

A basement membrane inter-separates the cells of the vascular component on most of their surfaces, but gap junctions have been demonstrated between different cell types of the vascular compnent of the juxtaglomerular apparatus(Boll, Forssmann and Taugner., 1975) as well as the intraglomerular and extraglomerular mesangial cells (Pricam, Humbert, Pesselet and Orci, 1974) . This finding raised the possibility of electrical coupling of the cells of the vascular pole of the glomerulus and the cells of the glomerular tuft (Barajas, 1979) .

Oberling and Hatt(1960) studied the ultrastructure of these cells in the rat and found that their cytoplasm appeared devoid of dense osmiophilic membrane-bound granules characteristic of the granular cells , but they contained some mitochondria, Golgi membranes, and sometimes small dense cytoplasmic bodies. As well,

they had some fibrillar bundles and attachment bodies at the cell membrane ; furnishing evidence for a relationship to some muscle cells. The latter authors added that these cells had cytoplasmic projections and formed a complex network at the hilus of the glomerulus.

In human juxtaglomerular apparatus, complexes formed by ordered crystalline material associated with droplets or granules and irregular granular material have been found . These granules did not correspond to the specific granules seen by light microscopy(Barajas, Sampson and Latta, 1965) and, instead, they resembled lipofuscin (Biava and West, 1965) . The latter authors stated that these lipofuscin- like complexes have also been found in granular cells and mesangial cells (Barajas and Latta, 1967) , and in smooth muscle cells of arteriolar walls in other human tissues (Biava and West,1965).

Oberling and Hatt (1960) and Bucher and Reale (1961) concluded that a relationship between the granular and agranular cells might exist as indicated by their similarities in fine structures, as well as by the observation of intermediate forms

and the mixture of cell types. As well, bundles of fibrils and attachment bodies, characteristic of smooth muscle in the wall of arterioles, were seen in both types of cells.

The macula densa is a specialized portion of the distal tubule as it approaches the hilus of the glomerulus and is in contact with the vascular component of the juxtaglomerular apparatus. With the use of light microscopy, it is characterised by the accumulation of nuclei (Peter, 1907 and Zimmermann, 1933) that may be piled up or simply closer to each other than in the cells of the adjacent parts of the distal tubule, apparently due to the cells being narrower in the macula densa . Another distinctive feature of the macula densa by light microscopy , is that the Golgi apparatus is located between the nucleus and the basal or lateral regions of the plasma membrane (McManus,1943).

Histochemical studies have demonstrated marked enzymatic activity in the macula densa for glucose-6- phosphate dehydrogenase and 6-phosphogluconate dehydrogenase as compared to a very little activity in the adjacent distal tubule regions (Hess and Gross,

1959 and Hess and Pearse, 1959). The authors also reported on an increase in the enzymatic activity of glucose-6-phosphate dehydrogenase in kidneys with high renin content such as the ischaemic kidney in rate with unilateral renal hypertension, or after adrenalectomy. Conversely, a marked decrease of the enzymatic activity was observed in renin depleted kidneys of rats given large doses of deoxycorticosterone or saline (Hess and Gross, 1959) or in the contralateral kidneny in renal hypertension(Hess and Pearse, 1959) . More recently, the enzyme sodium - potassium adenosine triphosphatase (Na-K-ATP ase) has been histochemicaly demonstrated in parts of the distal tubule other than the macula densa (Beeuwkes and Rosen, 1975 ; and Beeuwkes, Shahod and Rosen , 1975) . The lack of Na-K-ATP ase activity in the macula densa probably means that there is a relative decrease of the enzyme in the plasma membrane of its cells. This might cause the cytoplasm of the macula densa cells to be more readily affected by the fluctuations in the amount of sodium and potassium ions in the tubular lumen, which in turn is consistent with the sensory role proposed for the macula densa .

Electron microscope studies have revealed that the mitochondria in the macula densa are short and often more ovoid in shape than in the adjacent tubular cells (Latta and Maunsbach, 1962 ; Barajas and Latta,1963 ; and Tisher Bulger and Trump,1968) . In the rat, the mitochondria are scattered throughout the cytoplasm , while in man the mitochondria and other cytoplasmic organelles are located between the nucleus and the basal plasma membrane. The interdigitations of the plasma membrane are less developed than in other portion of the distal tubule and only rarely enclose mitochondria. Cytoplasmic projections extend from the basal part of the cells of the macula densa at their site of contact with the vascular component and the basement membrane of the macula densa, in the contact area, fuse with that of the vascular component and appears continuous with the basement membrane that surrounds the granular and agranular cells .

The close anatomical relationship between the tubular and vascular components of the juxtaglomerular apparatus agrees with theories that assign this structure a role in renal autoregulation. It inspired the

early histologists to suggest that the juxtaglomerular apparatus was involved in the regulation of the glomerular circulation and is a key component of the elaborate and experimentally supported theories of renal autoregulation proposed by Thurau (1964) ; and Guyton, Langston and Navar (1964) . In the same connection, the " macula densa hypothesis " for the control of renin secretion is resting heavily on the close anatomical relationships between the tubular and vascular components. This hypothesis was propounded by Goormaghtligh (1939 and 1945) who proposed that the macula densa might act as a sensor to regulate renin secretion by the juxtaglomerular granular cells. Later studies established that the juxtaglomerular cells are the site of synthesis and storage of renin . Physiologic experimentation, starting with the work of Vander and Miller (1964), using diuretics are consistent with the concept that the macula densa is involved in the control of renin secretion (Davis and Freeman, 1976) .

The functional considerations of the juxta - glomerular apparatus have stimulated anatomists to investigate the precise morphological relationship between its tubular and vascular components.

Elucidation of the anatomical features of this structure is likely to cast light on its possible role in tubuloglomerular feedback and on the mechanism by which the macula densa affects renin secretion. Contact of the distal tubule with the afferent arteriole and the extraglomerular mesangium (Lacis, Polkissen) was accepted and some authors described occasional contact with the efferent arteriole (McManus, 1942) . DeCastro and De la Pena (1952) are considered the only investigators who illustrated such a contact and called it an " atypical juxtaglomerular apparatus " . In the late 1950s , it was generally believed that the main feature of the juxtglomerular apparatus was the contact between granular cells in the afferent arteriole and the distal tubule (macula densa) .

The hard plastic, in which the tissue is embedded for electron microscopy , also facilitates cutting thin sections for appropriate staining and study with the light microscope. These technical improvement led to a detailed investigation of the tubulovascular relationships at the hilus of the glomerulus in the rat using serial semithin sections of plastic-embedded

tissue stained with to luidine blue(Barajas and Latta, 1963) .

Free hand clay reconstruction of several juxtaglomerular apparatuses were made from the light microscopic photographs. A new observation shown by the reconstruction was a long and constant association between the distal tubule and the efferent arteriole. In contrast, only a short contact between the distal tubule and the afferent arteriole was observed. Detailed studies revealed that the juxtaglomerular granular cells were predominant in the afferent arteriole, but were present in lesser numbers in the efferent arteriole. These observations have been confirmed with the electron microscope and supported the involvement of the efferent arteriole as a part of the juxtaglomerular apparatus(Barajas, 1979) .

A light microscopic study (Faarup, 1965) confirmed the obligatory contact of the distal tubule with the efferent arteriole and also described contact between the distal tubule, in its intercalated part, and the afferent arteriole. However, the macula densa

was found to be approximately equal in its contact with the efferent and afferent arteriole. The difference between the two studies may be due to the fact that Faarup (1965) studied the subcapsular and juxtameduallary nephrons, whereas Barajas and Latta(1963) studied only the superficial nephrons (Barajas,1979).

In the same context, Christensen, Meyer and Bohle (1975) studied the tubulo-vascular contact in man at the light microscopic level. They concluded that the amount of contact of the macula densa with the afferent arteriole was twice that of the efferent arteriole and stated that there was a substantially more contact of the macula densa and afferent arteriole than reported for the rat . Although species difference might be responsible in some measure for the more extensive contact reported in man, technical factors might also play a role. (Barajas, 1979). Thus, light microscopy provides a general topographic view of the juxtaglomerular apparatus, but the analysis of the degree of contact involves the clear visualization of basement membrane and all processes that require the resolution of the electron microscope(Barajas,1970).

Serial sections made from electron microscope studies have shown that the contact of the tubular and vascular components was of two types (Barajas, 1979) . In one type, observed in the extraglomerular mesangial region and the beginning of the efferent arteriole, the basal portion of the macula densa presented irregular cytoplasmic projections towards the vascular component. In addition, the basement membrane of the macula densa appeared continuous with that of vascular component with formation of a net work. The second type of contact, consisting of a simple apposition of the basement membranes , was observed most frequently between the distal tubule and the glomerular arterioles .