ABSTRACT

Ph.D. J.M. Rojo-Ortega, M.D. Investigative Medicine (Clinical Research Institute of Montreal)

The juxtaglomerular complex in various experimental states : Light and electron microscopic studies.

Modifications of Helly's and of Stieve's fixatives, and of Bowie stain demonstrate with excellent results the components of the JG complex.

A marked activating effect results by the combined addition of BaCl and $CoCl_2$ for the histochemical demonstration of the hexosemonophosphate shunt enzymes. A semi-quantitative index was derived for assessing the activity of these enzymes in the macula densa (MDl), with parallel measurements of the juxta-glomerular cell granularity (JGI) and renin.

Complete aortic ligature between the two renal arteries in rats over 300 g consistently produced atrophic left kidneys accompanied by hypertension.

JGI and MDI studies on sex and various strains of rats did not reflect significant differences. However, a zonal distribution of these two parameters was observed. In dogs and beavers, a similar distribution of the JGI was also encountered.

Morphologic studies revealed that JG cell granules appeared to be formed in the same manner as in other secretory cells. However, the release of the granular content seems to be different. Experimental degranulation states accompanied by an increase in plasma renin activity are described.

A morphological and possible functional independence between the JG complex components is pointed out. However, both JG cells and macula densa appeared to play an important role in sodium balance.

Electron microscopic studies demonstrated that JG cells are derived from arteriolar smooth muscle cells and have a rich nerve supply, possibly of adrenergic nature.

Studies in the "endocrine kidney" and severe renal ischaemia strongly suggest that the tubular fluid via the macula densa does not play a major role in JG cell granularity and renin release.

Short title: Dr.J.M. Rojo-Ortega Ph.D.

THE JUXTAGLOMERULAR COMPLEX

THE JUXTAGLOMERULAR COMPLEX IN VARIOUS EXPERIMENTAL STATES : LIGHT AND ELECTRON MICROSCOPIC STUDIES.

José-Manuel Rojo-Ortega, M.D.

Submitted to the Faculty of Graduate Studies and Research of McGill University in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

Department of Investigative Medicine
McGill University, Montreal.

Studies carried out in the Clinical Research Institute of Montreal and in the "Unité de Recherches de Pathologie Cardio-vasculaire de l'INSERM (Paris).

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Nature keeps whatever she
has done best closed, sealed until
it is regarded with reverence.

John Ruskin
in "Modern Painters" III.

To my parents and brothers.

IN HOMAGE TO MY TEACHERS.

Prof. Carlos Jimenez Diaz †

Director of the Institute of Clinical and Medical Investigations.

Madrid.

Prof. Fernando de Castro †

Chairman of the Histology Department of the Faculty of Medicine.

Madrid.

Prof. Jacques Genest.

Director of the Clinical Research Institute of Montreal.

Montreal.

Prof. Hans Selye.

Director of the Institute of Experimental Medicine and Surgery.

Montreal.

Prof. Pierre Yves Hatt.

Director of the Research Division of Cardiovascular Pathology.

Paris.

Prof. Angel Ortega Nuñez.

Head of the Clinical Section of the Beneficiary Provincial Hospital.

Madrid.

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LIST OF ABBREVIATIONS

Adrex	Adrenalectomy
Ao-lig	Complete aortic ligature between the two renal arteries
ATP	Adenosine-triphosphate
CM	Corticomedullary region or juxtamedullary cortex
DCA	Desoxycorticosterone acetate
DPN	Diphosphopyridine nucleotide, or Nicotinamide Adenine Dinucleotide (NAD)
E.K.	"Endocrine kidney"
Ep	Erythropoietin
GFR	Glomerular filtration rate
GPD	α- glycerophosphate dehydrogenase
G6PD	Glucose-6-phosphate dehydrogenase
HMP	Hexosemonophosphate
JG	Juxtaglomerular
JGC	Juxtaglomerular cells
JGCC	Juxtaglomerular cell counts
JGI	Juxtaglomerular granulation index
LC	Lacis cells
LRC	Lateral renal cortex
MDI	Macula densa index of glucose-6-phosphate dehydrogenase
MRC	Medial renal cortex
OC	Outer cortex
PAS	Periodic acid Schiff
6PGD	6-phosphogluconic dehydrogenase
PRA	Plasma renin activity

TG	Transitional granulated cells
TPN	Triphosphopyridine nucleotide
TPND	Triphosphopyridine nucleotide diaphorase
TPNH	Reduced triphosphopyridine nucleotide

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INTRODUCTION

Just over four decades ago, when Ruyter (1) described the presence of specialized granular cells in the afferent arterioles of the renal glomeruli, a new field of investigation was opened.

For many years, studies of the juxtaglomerular (JG) complex have been mostly related to hypertension. However, in the past few years, a new area of investigation was open by the demonstration of the production of "renin" by the JG complex and, by morphological, and physiological realtionship between the adrenal glands, sodium balance and the renin-angiotensin system.

This thesis is divided into two parts. The first consists in a comprehensive review of the anatomy and physiopathology of the juxta-glomerular complex.

The second part is devoted to the report of our experimental research work during the last 3 years, it is divided into six chapters. Much of the previous work in this area has consisted of isolated observations either on the JG cells, the macula densa, or the pressor activity of kidneys homogenates or of blood. Also the correlation of many data has resulted in different and at times confusing interpretations.

We have devoted our efforts to a comprehensive study of :
a) the histological and histochemical techniques used for the study of the JG complex, b) of the histology of the JG complex by light and electron-

microscopy with simultaneous measurements of the degree of JG cell granularity, c) of the glucose-6-phosphate dehydrogenase activity of the macula densa by devising a macula densa index.

The recent development of a sensitive and reproducible micro-method for the determination of plasma renin activity in rats by Dr. Boucher et al of the Institute has allowed us to carry out a more complete survey of the relationship between the juxtaglomerular components and peripheral renin activity.

PART I : REVIEW OF THE LITERATURE.

A - HISTORY

The first description of a specialized structure of the glomerular afferent arteriole was given by Ruyter (1) in Amsterdam, who in 1925 described what he called "a queer part of the afferent vessels". Ruyter showed that the smooth muscle cells in the distal part of the afferent vessels are replaced by one or more layers of epithelioid cells, many of which are granular, and that similar cells can be found more proximally, even in the interlobular arteries. Although these cells were more prominent in the mouse, he also found them in the rat, but not in the guinea pig, cat, dog, monkey or man. Rüyter compared these cells to those described by Schumacher (2) in the arterio-venous anastomosis of the glomus coccigious and he suggested that in the same manner the epithelioid cells of the afferent glomerular arteriole may play a role in the regulation of the local blood flow. Furthermore, using the Bielchowsky method, he found numerous fibrils, suggesting the presence of a rich nerve supply.

In 1927, Oberling (3) described in human kidneys the presence of these epithelioid cells in the media of the preglomerular arteriole filled with fuchsinophil granules, using the Masson's trichrome stain. He considered that these elements resemble the myo-epithelial cells described by Masson (4) in the digital glomus and derived tumors. Furthermore, he considered that the epithelioid cells had a rich innervation. Therefore, Oberling formulated what he called "housse neuro-musculaire des artères glomérulaires de l'homme".

In 1929, Okkels (5) reported granular epithelioid cells in the vascular hilus of the renal glomeruli of the frog. He also found these cells in the glomerular transitional zone between arteriole and capillary.

Okkels and Peterfi (6) stimulating the different vascular segments demonstrated that the glomerular capillaries do not respond. However, the neuro-myo-arterial apparatus responds to the least excitation by a strong contraction. They concluded that this segment plays an important role in the local regulation of the blood flow.

In 1932, Goormaghtigh (7) also observed these structures in man and in cat kidneys, and confirmed its analogy with the arterio-venous anastomosis of the skin. He described near the epithelioid cells, a group of cells morphologically different which he called pseudo-meissnerian cells, that he compared to the tactile corpuscules of the skin. The author designated both structures as "Les segments neuromyoartériels juxtaglomérulaires du rein".

In 1933, Zimmermann (8) did not exactly confirm Ruyter's description, claiming that near the glomeruli the arteriole wall represents a nodular group of 5 to 15 flattened cells in which the cytoplasm is occasionally light and without granulation that impinge on one side with the arteriolar endothelium and on the other, they lie back on the distal convoluted tubule. He called this nodular group of cells "Polkissen" (polar cushion) and described at this level of the nephron the characteristics of the distal tubule that Peter had already described in 1907 (9). Moreover he introduced the term "macula densa" for that region of the distal tubule, that displayed an agglomeration of the tubular cells.

Although he did not find the granular cells described by Ruyter and Oberling and without knowing Goormaghtigh's description of the pseudo-meissnerian cells, he introduced two new aspects: the "polkissen" and its relationship with the macula densa.

Later, Goormaghtigh (10) introduced the term "neuro-myo-arterial juxtaglomerular apparatus", considering that it is constituted of two different structures confused by Zimmermann under the name "polkissen": the epithelioid cells of Ruyter and Oberling and the pseudo-meissnerian cells that he described. The first is situated in the wall of the afferent arteriole, while the other is localized in the space situated between the glomerular arterioles. Both structures had a close contact with the macula densa. Furthermore, he considered the macula densa as a sensorial epithelium, by which the changes occuring in the distal part of the nephron would be transmitted to the afferent arteriole.

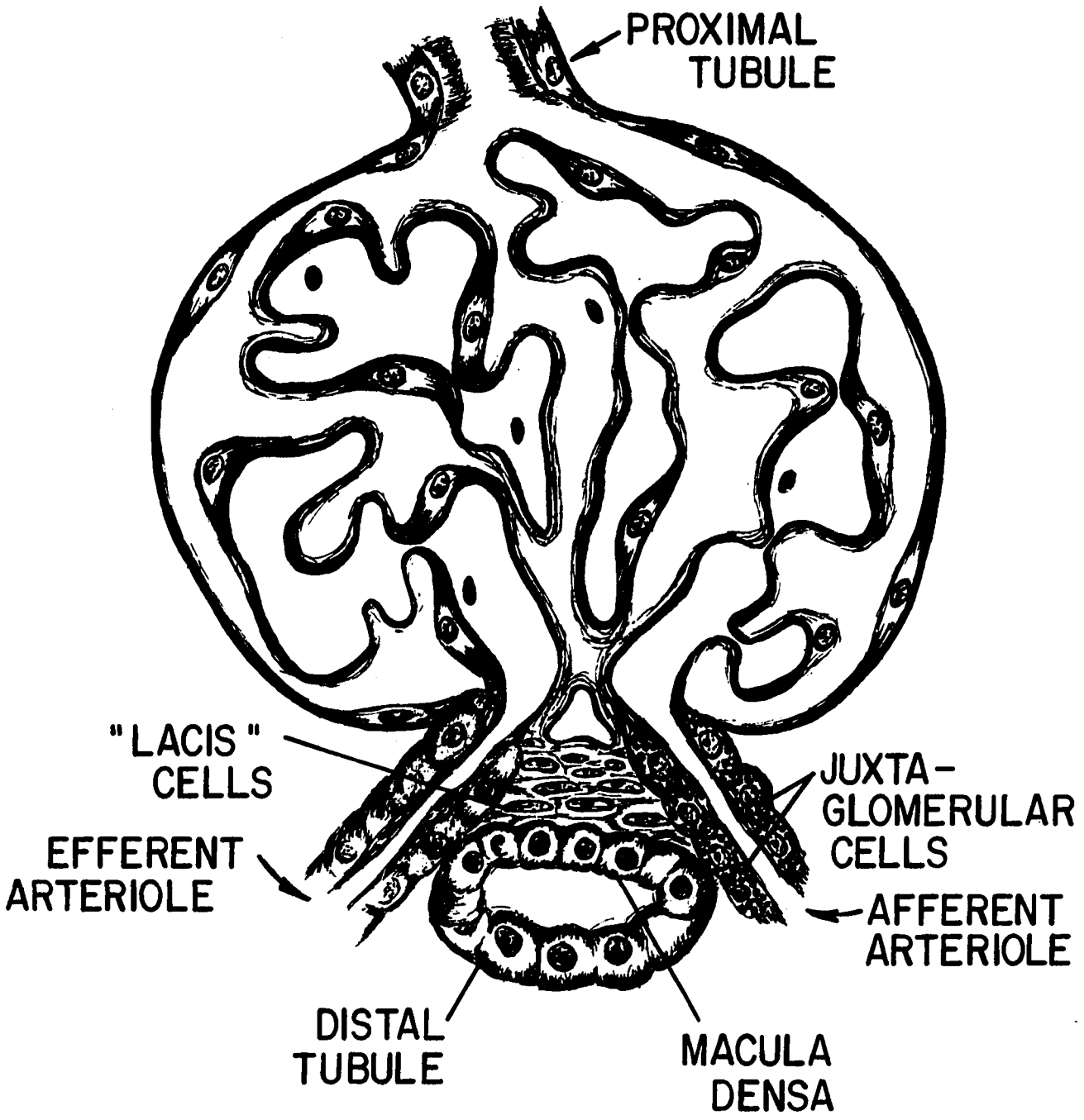
Goormaghtigh (11,12) suggested the term "juxtaglomerular apparatus" to include the epithelioid cells of the afferent arteriole, the macula densa, and the pseudo-meissnerian cells. Oberling and Hatt (13,14) demonstrated the precise structure of the macula densa and the pseudo-meissnerian cells, describing the latter as a cellulo-conjuntive lace-work or "Lacis". McManus (15), introduced the term "juxtaglomerular complex" which he felt was more apt in describing these structures. Barajas and Latta (16) included also the efferent arteriole in the complex due to its close relationship with the formulated structures and to the presence of granular cells in adrenalectomized rats.

B - DEFINITION OF THE JUXTAGLOMERULAR COMPLEX.

A perusal of the extensive literature describing the juxtaglomerular complex leaves the firm impression that the juxtaglomerular complex is considered to consist of the following structures: a) The granular epithelioid cells (also called juxtaglomerular cells (JGC), glomus or myo-epithelial cells) of the afferent and efferent glomerular arterioles. (In the efferent arteriole there is still too much controversy).

FIG. I

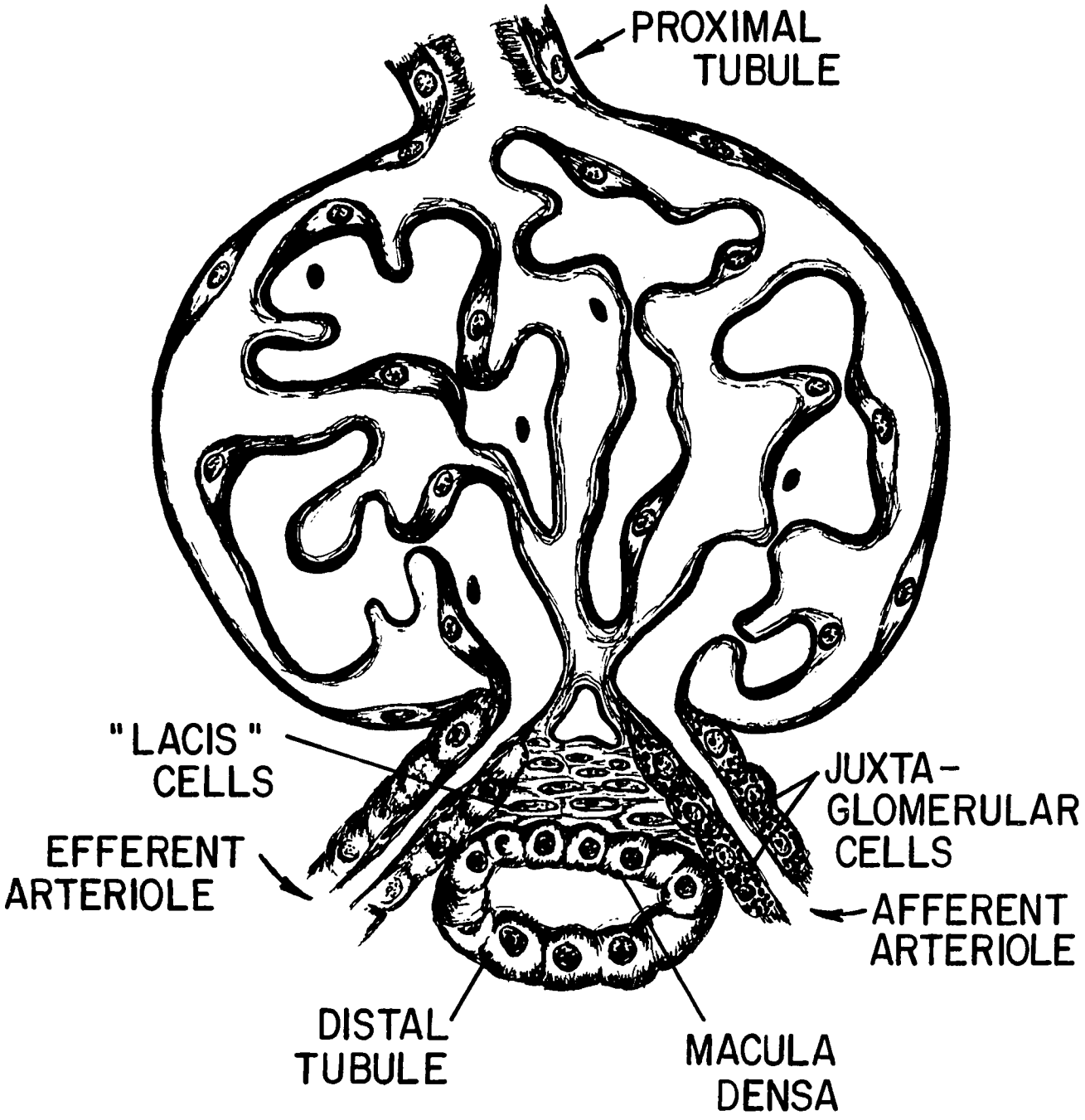
JUXTAGLOMERULAR COMPLEX



FROM HAM. A.W. (499)

FIG. I

JUXTAGLOMERULAR COMPLEX



FROM HAM. A.W. (499)

b) The "Lacis cells" (LC) or "cellulo conjuntive lace-work" of Oberling and Hatt (13,14) (also called pseudo-meissnerian "afibrillar" cells of Goormaghtigh or most of the cells of the "Polkissen" or polar cushion of Zimmermann). c) The macula densa (MD) of the distal convoluted tubule. The figs. 1 and 12 illustrate these main elements.

C - GRANULAR CELLS

1) HISTOLOGICAL PROCEDURES FOR STAINING THEIR GRANULES.

The identification of JG cells by light microscopy is difficult in paraffin sections stained by routine methods. In sections stained with hematoxylin and eosin, JG cells may be indistinguishable from smooth muscle cells of the arteriolar media except when they are enlarged, or hyperplastic and assume an epitheloid form.

Several histological procedures have been employed to demonstrate the granules of the juxtaglomerular cells and they are summarized in tables 1 and 2.

The most widely used staining procedures have been the periodic acid-Shiff technique (17, 18, 19, 20, 30), Bowie stain (21, 22, 23) and Gentian violet (24, 25, 26). However, it should be considered that these staining methods are dependent upon the fixative employed. Zenker-formol variant (Helly's fluid) (27, 28, 29), is generally considered the choice fixative.

Vital staining methods have also been employed for demonstrating JG granules, such as neutral red (25, 36, 76) Acridine-orange, Brilliant cresyl violet, Nile blue sulphate (76) or the mixture of Brown of Bismarck with neutral red or Nile blue (37). With thioflavine T fluorochrome it has been shown that JG cell granules become fluorescent (38, 39).

For light microscopic demonstrations of the granular cells from tissue embedded in plastic resin, various methods have been described using for

TABLE 1

Histological procedures for staining granules of the JG cells.

Authors	Fixatives	Stains	Results
Ruyter (1)	Kopsch!s fluid	Altmann	+
	Maximow's fluid	Kull	+
	Zenker-Formol	Iron hematoxylin	+
		Sudan III	+
		Blue Nil sulfate	+
		Osmic acid	+
Goormaghtigh (10, 11)	Bouin - Holland	Masson's trichrome	+
	Zenker-Formol	Hemalum, phospho-tungstic acid	±
		Light green	
		Hematoxylin - Erythrosin	±
Dunihue and Candon (27)	Zenker-Formol	Goldner's modified trichrome	+
Namiki et al (24)	Zenker-Formol		
	Formol 20%	Gentian violet	+
McManus (17, 18, 30)	Cobaltous-formalin Calcium	Trichrome	+
	Cobaltous-formalin Calcium	Sudan black	+
	Bouin	Periodic acid Schiff	+
Wilson (31)	Bouin - Formalin	Crystal violet	+
	Zenker-Formol (Helly's fluid)	Ethyl violet	+
Hartroft and Hartroft (32)	Zenker-Formol (Helly's fluid)	Bowie	+
Grzycki (33)	Zenker-Formol	Trichrome	+

* (-) no stain.
(±) inconstant stain.
(+) regular stain.

TABLE 2

Histological procedures for staining granules of the JG cells.

Authors	Fixatives	Stains	Results
Harada (25, 26)	Harada's modification of Ciaccio's fluid	Goldner's modified trichrome	+
		Borster-Vines	+
	Kolatchev	Kolatchev for Golgi apparatus	+
	Champy	Altmann for mitochondrias	+
	Formol	Dietrich-Smith	+
		Saguchi-Marusawa	+
	Carnoy	Methyl-pyronin green	+
		Gentian violet pH 7	+
		Methyl violet, crystal violet	+
	Ciaccio, Lison	Thiazin (methylene blue, thionin, toluidin blue)	+
		Hempelmann method	+
		Omo's toluidin blue method pH 7	+
		Azines (Brilliant cresyl blue, nile blue sulfate) and	+
		Oxazines (neutral red, safranin)	+
		Best Carmine	
		Muci-Carmine	±
Endes (34)	Formol	Phosphotungstic acid hematoxylin modified	+
Hennebert (28)	Bouin	Chromic hematoxylin Gomori phloxine	+
	Bouin - Allen	Bauer for mucopolysaccharides	+
	Zenker-Formol (Helly's fluid)	Bowie	+
Hatt et al (35)	Stieve's fluid	Alcian-Blue PAS	+
Friedberg (49)	Harada's modification of Ciaccio's fluid	Bowie	+

that purpose the thick section (about 1 μ) cut with an ultra-microtome (40, 41, 42). However, the small size of the specimen does not allow semiquantitative analysis.

a) Juxtaglomerular cells and mast cells. Juxtaglomerular cells contain granules which, under certain conditions, stain metachromatically. These elements have been thought to be modified mast cells. Harada (25) observed that the granules of the JG cells stain metachromatically with methyl violet, crystal violet and dahlia violet. Corbascio (43) claimed that the juxtaglomerular cells of the rat kidney have many characteristics in common with mast cells. Their granules stain metachromatically with certain dyes to which mast cells granules react in the same manner. Furthermore, the granules of the JG cells are discharged under the influence of dextran or ovomucoid as are those of the mast cells. However, Demopoulos et al (44) have provided convincing evidence that the juxtaglomerular cells do not stain metachromatically under usual conditions. These authors have shown that after fixation in strong oxidants such a potassium-dichromate, the JG cells can stain metachromatically. However, these cells, unlike the mast cells, do not discharge their granules under the influence of 48/80 or dextran, nor do they stain metachromatically under ordinary conditions and hence, they are not considered to be mast cells.

As described in the following paragraphs, the structure of the juxtaglomerular cells eliminate any similarity with the mast cells.

2 - MORPHOLOGICAL DESCRIPTION

a) Light microscopy. By light microscopy, as first described by Ruyter (1) the JG cells are recognized chiefly by the presence of stainable

granules and by their location in the wall of the afferent arteriole usually near its entrance to the glomerulus. These observations have been confirmed by many authors (3, 7, 15, 45, 46, 47). However, they have been observed distal to the glomerulus and in the interlobular arteries (1, 48, 49). Furthermore, in the efferent arteriole, Barajas and Latta (76) have found granular cells in adrenalectomized rats, Hartroft (53) in dogs with a sodium deficient diet, Adebahr (50) in human kidneys after intoxication by E 605 (a plant antiparasitic). Similar findings were reported by Monserrat in man (51, 52). As it was pointed out by Ruyter, these cells have an epithelioid nuclei, (round or oval) and an absence of myofilaments. Harada (25) has shown in the mouse using histological techniques for mitochondria, that the JG cells' mitochondria appear as fine granules, mostly near the nucleus. The secretion granules are much larger and more numerous, confirming the observations of Goormaghtigh, (10, 12).

Harada (25) also described by using an osmium method, that the Golgi apparatus of the JG cells varied in size and shape from one cell to another and is located between the lumen of the arteriole and the nucleus. Hennebert (28) has observed, using osmium and silver techniques, that the Golgi apparatus is more developed in the granular than in the agranular stage.

b) Electron microscopy. With electron microscopy the cytologic characteristics of granular JG cells have been further elucidated. In various species most of the granules appear relatively homogenous, dense and osmophilic, (54, 55). Oberling and Hatt (13, 14) observed the granules to be single or membrane-bound. This was later confirmed by others (56, 57, 58). Bucher and

Reale (58, 59) have described that the granules seem to be formed by a fine granular structure that they called "granula in granulis". Similar findings were reported by Hartroft and Newmark (56). Later, Chandra et al (61, 62) confirmed this observation and they considered that the fine granules or filaments of about 150 A^{o} in diameter might represent aggregates or subunits approximately 40 A^{o} in diameter. Structure and shape heterogenicity of the granules have been observed by various authors (57, 61, 63) and it has been implied that it may represent different stages in the formation of granules (14, 62, 66). Chandra et al (62) have reported that frequently granules were not completely limited by membranes. Lysosome-like forms have been observed in JG cells of several species (16, 62, 68). Positive acid phosphatase activity was first demonstrated by Lee and co-workers (67, 68), and later confirmed by Fisher (69). Complex bodies or lipofucsin-like granules have also been seen in human JG cells (79, 80). Barajas and Latta (16) in one electron-micrograph (No 10) demonstrated in adrenalectomized rats a type of granule resembling lipofucsin droplets. The cytoplasm contains a moderate number of short or round mitochondria, a moderate amount of endoplasmic reticulum, considerable number of ribonucleaprotein particles, centrioles and a Golgi apparatus mainly formed by lamellar sacs and small vesicles, (13, 14, 56, 57, 60, 62, 64). Bundles of myofilaments have also been described (14, 56, 57, 66).

3 - MORPHOLOGIC EVALUATION

Since by experimental conditions or pathological states the granularity of the JG cells may vary, therefore, various counting methods have been devised in order to measure semiquantitatively their degree of granularity.

The most common methods employed are the juxtaglomerular granule indices (JGI) devised by Hartroft and Hartroft (32, 71) and Dunihue and Robertson (72). Furthermore another counting method mostly used in man is the juxtaglomerular cele counts (JGCC) described by Turgeon and Sommers (73) by which they estimated the degree of granularity and also the relative total number of cells at the vascular poles of the glomeruli (granular and agranular cells). Naturally all of these methods depend to some extent on the problem of methodology and subjective criteria. However, they are very useful for a trained person when a semi-quantitative estimation of the JG cell granularity should be done among groups of experimental animals or human kidneys, allowing statistical evaluation with other parameters.

4 - HISTOCHEMICAL NATURE OF THE GRANULES.

Few histochemical studies have been done in order to elucidate the composition of the juxtaglomerular granules as it has been described above. Ruyter (1) demonstrates a sudan III positive test. McManus (17, 18, 30) showed that the granules give a periodic acid Shiff (PAS) and sudan black positive reactions. Harada (25) found a lipid and ketosteroid protein complex, as well as ribonucleic acid components in the granules, and suggested that the secretion of the cells are related to acid mucopoly-saccharides. Gomba et al (74) confirmed the presence of lipids and showed that the positive PAS test is mainly due to mucoproteins and glyco-proteins, which was confirmed by Kroon (20). Furthermore, these authors confirmed the presence of protein in the granules using tests for proteins accompanied by enzymatic digestion with pepsin and trypsin. However, after extraction with trichloro-

acetic acid and perchloroacetic acid the granules were well stained with the Endes trichrome and PAS indicating that the nucleic acids play no major role in their structure. They also found a negative Fuelgen and methyl-green pyronin test, in contradiction to the findings of Harada (25) who claimed a pyronin positive reaction. A negative (28) methyl-green pyronin test has been also reported by Hennebert. Later Gomba et al (75) have pointed out that the juxtaglomerular granules of albino mice contain tyrosin and a significant amount of tryptophan, describing also an alkaline tetrazolium reaction suggesting that the granules contain a reducing substance that may be of lipoid origin or consisting of reducing sugars. However, these authors failed to demonstrate the presence of sulphurous amino acids and arginine. Recently acid phosphatase has been found in the granules (67, 68, 69, 70).

5 - SECRETORY CHARACTERISTICS.

a) General considerations.

During early 1898, the hypothesis of muscle glandular cells was advanced by Gilson (81) in the worm where the nephridies would be atrophic. This observation was taken by Goormaghtigh who assumed that the epithelioid JG granulated cells are endocrine cells in nature (11, 82) having observed a secretory cycle in ischaemic kidneys of rabbits. In a first step the secretory granules are small, later they become larger and more numerous, then in the cell some vacuoles appear containing granules and finally the vacuolization is increased, the granules disappear giving the cell cytoplasm a light and homogenous aspect.

Goormaghtigh (12) further postulated, although evidence was lacking that the secretory product was the pressor substance "renin".

Hennebert (28) found, using a special method for demonstrating the Golgi apparatus in light microscopy, that hypergranulated cells of rabbits infected with taenia pisiformis, presented an enlarged Golgi apparatus, as compared to other lesser granulated cell states. This author also found vacuoles, sometimes containing granules.

Light epithelioid vacuolated cells have been also described by Hartroft (53) in the dog and by Turgeon and Sommers (73) in man.

Miller and Hartroft (83) have reported that acute sodium restriction for a few hours in rats produces a degranulation of the JG cells followed by a regranulation later on. They also found that a similar pattern followed the renal renin content. However, plasma renin activity was not measured.

With electron microscopy, the secretory characteristics of the juxtaglomerular granulated cells have been further elucidated. The juxtaglomerular cells in all species studied have features suggesting a high rate of protein synthesis and secretory activity, relatively abundant mitochondria, endoplasmic reticulum with ribosomes, Golgi elements and osmophilic (secretory) granules bounded by distinct membranes.

Furthermore, an increased plasma renin activity, accompanied by a degranulation of the JG cells and an increase of light vacuolated epithelioid cells, has been observed in dogs after acute ureteral clamping of the left kidney (201).

b) Granule formation.

The origin of the juxtaglomerular cell granules from the Golgi apparatus was first suggested by the work of Barajas and Latta in a study of adrenalectomy in rats (16) and has been observed subsequently in a variety of species and experimental conditions. The development of juxtaglomerular granules has been studied in rats, monkeys and human kidneys, including those from patients with renovascular hypertension, and those from rats with constricted renal arteries (77, 80, 86), in cats, rabbits and rats, including sodium deficiency (56, 62) and in mice, after hypertonic intraperitoneal fluids, or cobalt nitrate injections (67, 68).

Chandra et al (62) have shown different sizes of granules in the Golgi zone of the JG cells, and they suggested that the storage of the synthesized material and the maturation of the granules take place in the Golgi apparatus. Furthermore, the same authors have shown that in rats with a sodium free diet, a dilated endoplasmic reticulum occurs containing a dense material similar to the granules. They also observed in some sections a continuity of the distended endoplasmic reticulum with the limiting membrane of the granules. Thus, a cycle of synthesis in the juxtaglomerular cells of the rat was proposed by Chandra et al that follows the general pattern observed in other secretory cells (pancreas, gastric mucose, thyroid).

Barajas (78) has described that within the cisternae of the Golgi apparatus, the earliest forms of JG granules, or protogranules, exgibit a crystalline appearance. He suggested that the mature granule seems to be formed by coalescence inside the smooth-surface membranous sacs of multiple protogranules

which retain their crystalline pattern in the conglomerates, or by progressive growth of a single protogranule. However, it should be considered that differences in the structure of the granules' forms have been noted (see comparative anatomy).

Dilated endoplasmic reticulum of the JG cells has been generally observed in a variety of species, including man, under physiological or pathological conditions in which the granularity of the JG cells was usually increased (14, 16, 65, 86). However, dilated endoplasmic reticulum has also been encountered in some hypogranulated states, such as after injections of cobaltous nitrate of hypertonic peritoneal dialysis (67, 68).

Barajas and Latta (16) have noticed in adrenalectomized rats that the amount of Golgi membranes was difficult to estimate, but it seemed that cells with few granules had tended to have a large Golgi complex and, conversely, cells with many granules tended to have a small Golgi complex. On the other hand Fisher et al (70) found prominent Golgi structures in JG cells packed with many granules in ischaemic kidneys.

Hinglas et al (86) have observed a hypertrophy of the Golgi apparatus and numerous lysosome-like forms in the JG cells of the contralateral kidney to the clamped one in rats. They suggested that the degranulation process of the unclamped kidney may occur in situ by degradation of the granular content and lysosomic formation.

c) Granule secretion.

The ultrastructure of a cell which is active in synthesis and secretion has been described in many organ systems and experimental situations. The

accepted concept is that the secretory material is synthesized on the ribosome template in the endoplasmic reticulum and conveyed to the Golgi apparatus, where it accumulates in vesicles until it eventually becomes separated and appears in the cytoplasm as a secretory granule (87-98). Such a secretory granule is limited by a single unit membrane and usually leaves the cell by fusion of its membrane with the cell membrane (92, 97, 98). The granules of the juxtaglomerular cells appeared to be produced in this way, but the secretory material apparently is extruded from the cell in a different manner.

Reale et al (99) found in JG cells of mice, the presence of vacuoles containing some granular material similar to the granules. Therefore they suggested, based on Goormaghtigh's (12, 82) and Hennebert (100) observations, that vacuoles may represent the large empty granules that have once extruded the secretory material.

Lee et al (67, 68) who gave special attention to this subject have reported in mice, that peritoneal dialysis with hypertonic dextrose, as well as subcutaneous injections of cobaltous nitrate produces an acute discharge of granules followed by a regranulation process as measured by the JGI. During the degranulation period (mostly in cobalt treated animals), these authors claimed to observe that the coalescence of granules was increased, and that electro-dense material resembling a granular content was occasionally noted in the intercellular spaces. Until now no data concerning the fusion of membranes have been encountered as far as we know.

It should be noted that in Lee's experiments plasma renin correlations were done.

Furthermore, a cytotoxic effect of cobaltous salts on the kidney (28, 100) has been reported in various animal species and in pancreatic alpha cells (101, 102) even with smaller doses than used in mice by Lee et al (68).

It has been postulated that the presence of nucleoside diphosphatases in the membranes of secretory granules, as in the anterior pituitary (103, 104), provided high energy phosphate bonds which supply the energy necessary to extrude the secretion granule from the cell.

Lee et al (70) have reported an absence of inosine dephosphatase in the granules of the JG cells, suggesting that the biochemical mechanism of granule secretion differs from that of other secretory glands.

It appears from the foregoing review that the JG cells are secretory in nature. However, the mechanism of discharge of granules appears to be different from other secretory cells.

6 - NATURE OF THE ELABORATED PRODUCT.

The elaboration of two secretory products by the JG cells is a possibility at the present time - first, the enzyme renin and, secondly erythropoietin or erythrocyte stimulating factor. Evidence in favor of the latter is associative and open to question, whereas that in favor of renin is both voluminous and more convincing.

a) Renin. Two swedish scientists Tigersted and Bergman (105 in 1898) demonstrated that a saline extract of the rabbit's kidney was capable of

producing elevated pressure in animals. They named this material "renin". Subsequent work (106-112) has shown that renin acts as an enzyme in the conversion of a plasma substrate, an alpha-2-globulin to produce a decapeptide, now called angiotensin I. Alone, this decapeptide has virtually no vasopressor activity, but in the presence of an enzyme (converting enzyme) which is widely distributed in body tissues, including plasma, it is rapidly converted to an octapeptide, called angiotensin II, which is an extremely potent vasopressor substance. The molecular weight of the unpurified renin seems to be from 40.000 to 49.000 (120). The molecular structure of both angiotensin I and II has been established and synthesized (113 - 119). In addition this enzyme system has been shown to play a role in the control of aldosterone secretion (121 - 123).

There are several lines of evidence indicating that "renin" is concentrated in the vascular pole of the glomerulus.

Evidence for renin elaboration by JG cells first proposed by Goormaghtigh, was based on associative studies until recent years following his observations of renal ischaemia (125). Dunihue (126, 27) observed prominent and hypergranulated JG cells in hypertensive rabbits and agreed that the renin theory was reasonable. Others disagreed, however, claiming that JG cells had no relation to hypertension (127). Later, it became apparent,by correlation of various studies, that extractable renin was parallel to the degree of granulation of JG cells in animals with unilateral renal hypertension (128, 129, 130), in adrenalectomy (131, 132, 133) and sodium chloride and deoxycorticosterone acetate (DCA) administration (134, 32). The first direct correlation of these

two parameters were demonstrated by determining them in the same animals. Marshall and Wakerling (135) working on extracted hog renin have shown that this substance gave a Hotchkiss's periodic acid schiff (PAS) reaction, then the authors applied this reagent to a histological preparation of dog kidneys in which they measured the renin content. They found a good correlation between renal renin content and the degree of PAS reaction in the JG cells. They also reported that the solubility characteristics of renin and those of the granules of JG cells were identical.

Pitcock et al (136) demonstrated that when rats are placed on a low sodium intake, both the renin content and the JG granularity increase in their kidneys. Conversely, Tobian (137) has shown that rats on a high intake of sodium have a concomitant reduction of renin and JG granules. Tobian's group has reported that in rats with clip-hypertension the JG granules practically disappear in the contralateral kidney. At the same time, the amount of extractable renin in this contralateral kidney also becomes significantly small (138). If the "ischaemic" kidney is subsequently excised, both the JG granules and the extractable renin reappear in normal amount in the contralateral kidney (139). Similarly, if DCA and salt are given to rats JG granules practically disappear and there is a virtual disappearance of extractable renin (138). Conversely, Fisher (140) has shown in rats with clip-hypertension, that JG granularity and extractable renin runs parallel in the ischaemic kidneys. In patients with renovascular hypertension a parallel correlation was found in the damaged kidney between renal renin content and JG granularity (141, 142). These findings have been confirmed and extended in a variety of experimental conditions. (140, 143, 144, 145).

Other evidence includes effects produced by renin and antibodies to renin. JG cells become hypogranulated in response to an exogenous source of renin (53, 146) and conversely, their response to antirenin, whether the result of active or passive immunity, is hypergranulation (53). In both cases a close correlation of renin with JG granulation was observed.

Cook and Pickering (147) using an ingenious technique for extracting glomeruli from other renal tissue by first injecting magnetic iron oxide intravascularly, showed that renin was present only in the vascular pole. Originally Cook et al (148) found that the renin content was correlated with the number of glomeruli in the outer third of the renal cortex, but in the middle and deeper layer renin was absent while the glomeruli were still plentiful.

By the use of the fluorescent technique for staining antibodies to antirenin, Nairn et al (149) found that most of the renin appeared to be on the glomeruli, probably the epithelial cells. However, Edelman (150) and Hartroft, and Hartroft (53, 151) utilizing a more precise technique have found that the juxtaglomerular cells of sodium deficient rabbits stained heavy for renin in contrast to the negative staining of macula densa. The same results have been demonstrated in the meso and metanephros of the embryo pig (152). Recently, Warren et al (153) using a more purified renin but with a more crude fluorescent antibody technique than that used by Hartroft's group, have found bright fluorescence mostly located at the vascular pole of the glomerulus, staining also heavily the JG cells and macula densa.

In microdissection studies, however, Bing (154) found only 50 per cent of the renin concentration of the vascular pole left after macula densa was dissected away. He later reported the presence of renin in the nephrogenic zone of newborn pigs in which there were typical macula densa but no glomerular arterioles (155, 156). He suggested that renin was formed in the macula densa and part of it was deposited in JG cells. Hess and Regoli (157) came to a similar conclusion when they found that an increase in enzymatic activity of the glucose-6-phosphate dehydrogenase of the macula densa and extractable renal renin preceded the increase in degree of granulation of JG cells in renal ischaemia. Endes et al (158) reported that kidney homogenates of newborn rats which contain no granulated cells, exert a pressor effect.

On the other hand, it has been pointed out that the distribution of renin is greatest in the outer zone of the mature renal cortex (148, 159) and so is granulation of JG cells (160, 48), whereas the macula densa appears the same in all zones (48). Furthermore, ischaemic kidneys with atrophic macula densa but hyperplastic JG cells renin is increased (143). In some types of fish (teleosts) in which the macula densa is not present, the JG cells are rich in granules (40, 161) and in renal renin content (161). Studies on ultra centrifugation of kidney homogenates gave more support to the presence of renin in such granules. Cook (162), Dengler and Reichel (163) claimed that the pressor activity is associated with granules approximating mitochondria in size. Chandra et al (61, 62) were able to isolate the pressor activity in a well defined "liquid pellet" which revealed on electron microscopy, fine granules of about 40 A° in diameter, that they

postulated to be subunit constituents of the JG cell granules. A new appreach to the problem may be provided by the work of Robertson et al (164, 165) who demonstrated the production of renin (as measured in the supernatant of the media) by cells containing Bowie positive granules in organ cultures of renal cortex. Since no evidence of Bowie positive granules has been reported in the macula densa, this also supports the concept that JG cells both produce and secrete renin.

It cannot be ruled out that the macula densa may play a role in the synthesis of renin, but the majority of evidence implicates JG cells as the primary site of both the manufacture and secretion of renin.

b) Erythropoietin. Since the establishment of a concept that erythropoiesis is controlled by erythropoietin (Ep) (166-169) a search for the production site of Ep has been one of major interest. From the results obtained from both clinical and experimental research carried out so far, the kidney has been regarded as the organ playing an important role in the production of Ep (170-174).

It has been pointed out by Osnes (175) that the potent activity of Ep in plasma obtained from adrenalectomized mice following phlebotomy is usually associated with an increase in granularity of the JG cells. Hirashima and Takaka (176) found hypergranulation of JG cells associated with an increase in erythropoietin in animals with renal ischaemia produced by renal artery clipping and with anemia produced by bleeding or phenylhydrazine. They also found that both hypergranulation of the JG cells and the activity of Ep in plasma showed a decrease in hypertransfused polycythemic rats. Mitus and Toyama (177) came to a similar conclusion with the rat by clipping the renal arteries. Luke et al (178) and Jones (179) have reported cases of renal hypertension and polycythemia.

In contrast, the reported failure of rats with hypertension induced by the clipping of renal arteries to show increased Ep secretion has been described by Cooper and Nocenti (195).

Kaley and Demopoulos (180) have reported an increased granularity of the JG cells in rat following cobalt administration or hypoxia exposure, stimuli known to increase erythropoiesis. Demopoulos et al (181) described changes in granulation of JG cells with changes in erythropoietin following both acute and chronic exposures to simulated altitudes under conditions in which blood volume and electrolyte changes were not altered.

On the other hand, Goldfarb and Tobian (182, 183, 184), attempted to separate the variables by using a simulated altitude chamber to produce hypoxia without a change in distension of the renal arteriolar bed and conversely, exposed rats to high oxygen concentrations. They found no change in the degree of granulation of JG cells in either case. Yazgan (185) came to a similar conclusion in mice, exposed to high CO_2 concentrations. No changes in JG cells were observed by Hollinshead (186) in mice exposed to hypoxia.

Reissmann et al demonstrated that poisoning with mercury chloride, (172) which produced acute tubular necrosis, abolished the animals ability to produce Ep as effectively as bilateral nephrectomy. They have proposed that renal tubular tissue constitutes the site of production of erythropoietin in the kidneys. Penington (187) reported in hog kidney that the erythropoietic activity was distributed almost equally between the cortex and medulla. However, renin was only found in the cortex. Furthermore, Fisher et al (188) by fluorescent antibody technique have reported the

localization of Ep in glomeruli of sheep kidneys.

The possible relationship between the renin-angiotensin system and the erythropoiesis has been explored by several authors. Fisher and Crook (189) reported that the administration of angiotensin II to hypophysectomized and hypophysectomized-adrenalectomized rats resulted in increased erythropoiesis without significant change in oxygen consumption. It was, however, reported by Bilsel et al (190) that no significant increase in erythropoietic activity was observed after the injections of Angiotensin II in amounts similar to those used by Fisher. Recently Nakao et al (191) reconsidered the problem and have described that two daily intravenous injections of angiotensin II have no effect on rabbit erythropoiesis. However, when given by intravenous drip over a 30 minute period, they found a significant increase in erythropoiesis as well as norepinephrine. The authors conclude that the increased erythropoietic activity of angiotensin II may have been induced by renal ischaemia. It should be pointed out that angiotensin II decreases in man (192, 193) and in dog (194) the plasma renin activity.

From the foregoing review it appears that the concept of erythropoietin being elaborated by the JG cells deserves consideration but more positive evidence is necessary.

7 - JG CELL GRANULARITY AND PLASMA RENIN ACTIVITY (PRA)

a) General considerations.

The widely accepted correlation between the renal renin content and the JG cells granularity, suggested to some investigators that the degree of granulation was representative of renin production. However, as in other

secretory cells (92, 93, 196) granularity can be regarded as representing a phase of storage of secretory products mainly, and an increase in the secretory activity of the cells would be expected to be associated with depletion of such granules. On the other hand, dense material in dilated endoplasmic reticulum, and small granules in early stages of their morphogenesis, such as those which can be detected by electron microscopy in the Golgi apparatus, constitute evidence of active formation of new secretory granules and therefore should reflect from the morphological point of view, more closely the activity of the cells in the synthesis of secretory products ("e.g." renin).

The content of renin in the kidneys gives no more than a slight indication of the secretion rate of the enzyme and does not permit any conclusions to be drawn on the concentration of renin circulating in the plasma and hence liberating angiotensin outside the kidney.

Since the experimental conditions may differ widely, acute experiments of a few hours duration, and experiments that run for several days or even months must be considered separately.

b) Acute experiments.

In acute experiments renin secretion does not always parallel the renin content or JG cell granularity. Thus, in an acute experiment such as sodium depletion by peritoneal dialysis, hemorrhage or thirst, renal renin is normal despite an increased pressor activity of the plasma of such rats (197, 198).

Fisher et al have reported in rats that after peritoneal dialysis, no significative changes occur in the JG cells granularity, (199). The first direct study of the JG cell granularity and plasma renin activity determinations in the same animals has been done in our Institute (200). No significant changes

in rats were found in JG cell granularity despite a marked increase in plasma renin activity in the experimental situation refered above. In contrast Miller and Hartroft (83) reported in an abstract that in weanling rats a sodium deficient diet for few hours produces a decrease in granularity followed by an increase in JG cell granularity, following a similar pattern, the renal renin content. Tremblay (201) claimed to observe that in dogs after acute ureteral clamping, a degranulation of the JG cells occurs following by an increased plasma renin activity in the renal vein. Taquini et al (202) have reported that acute partial ischaemia in dogs, increase both renal renin, and plasma renin.

c) Chronic experiments.

In chronic states, it has been postulated that a parallel exists between the JG cells and plasma renin activity (53, 203).

i) Sodium changes. If rats are kept for several weeks on a sodium deficient diet, the JG cells granularity, the renal renin content (136, 62, 208) and circulating renin are elevated (204). In human sodium depleted subjects, Azjen et al (205) have found a close correlation between renal vein renin and the JG cells. Conversely, in the rat a prolonged sodium load, either by diet or enhanced by the simultaneous administration of DCA, leads within several weeks to the disappearance of the JG cells granularity (32, 130, 135, 145) and renin from blood and kidneys (145, 206, 123, 207, 209).

ii) Adrenalectomy. In the rat, adrenalectomy followed neither by corticoid substitution nor by the administration of additional salt leads within a week to a marked increase in circulating renin (215) and in renal renin content (133). In contrast, Böhle (210) did not find in similar

conditions JG cell hypergranularity.. However, he observed a relative hyperplasia of epithelioid cells. Furthermore, Dunihue et all (211, 212, 213) and later on other authors (16, 214, 130) have observed in rats as well as in other animal species, a marked increase in JG cells granularity when adrenalectomy is followed by corticoid substitution or sodium supplement. In Addison's disease, a hypergranularity of the JG cells has been reported (216) as well as an elevated plasma renin activity (217). However, when the patient was therapeutically compensated the values of plasma renin activity became much lower than in the decompensated state.

iii) Grafting Kidneys. In the rat, by employing the technique of grafting kidneys (avoiding the period of ischemia) containing various amounts of renin into a nephrectomized test animal, it has been possible to show that the increased renin content of the kidney is in parallel with an elevated secretion rate, and correspondingly, that renin depleted kidneys do not secrete the enzyme (218, 219). By the same technique Masson et al (220) reported that in rats treated with DCA, salt or cortisol, the liberation of renin soon decreased after the onset of treatment while the renin content remained unchanged or decreased much later. Hennebert (28) by grafting renal cortex of rabbits found a relative hypergranularity of the JG cells. Similar experiments and results have been reported by Endes et al (221) in the rat.

iv) In renal hypertension. In the rat, an increase in the renin content (222) and JGC granularity (157, 223) of the clamped kidney is first demonstrable after 48 hours. However, this pattern was not followed by the contralateral kidney, which decreased more rapidly in renin content without decrease in JG cells granularity. Under similar experimental conditions it has

been reported that circulating renin levels run parallel to the increase of renin content (215). This differs somewhat with the dog, in which a definite increase in renal renin content (202), renin concentration of blood or renal lymph can already be observed within a few minutes up to 1 hour after the perfusion pressure has been reduced by a clip on the renal artery or by other means (202, 224-226).

Recently, from our Institute (141, 142) it has been reported that patients with renovascular diseases have a close correlation between renal renin content, JG cells granularity and plasma renin activity in the renal vein.

v) Secondary hyperaldosteronism. Very little evidence has been provided to correlate JGC granularity and plasma renin activity in secondary hyperaldosteronism and most of the data reported in the literature are indirect parameters. Davis et al (227) demonstrated that the renin content and JG cell granularity of dog kidneys with inferior vena cava constriction and ascites were significantly increased. In rats with aminonucleoside nephrosis, Tobian et al (228) found a correlation between the amount of ascites and the increase in JGI in rats. On the other hand, Gross et al reported that the renal renin content of rats with aminonucleoside nephrosis did not differ from that of normal rats. Nichols and Heanigar (230) reported that dogs infected with heart worms developed pulmonary hypertension accompanied by thickening of the adrenal zona glomerulosa increased granularity of the JG cells, ascites and edema. Gliedman et al (231) have shown, after division of the common bile duct in dogs, a marked augmentation of the JGI, and in some cases, of aldosterone.

Merril et al (232) found a significant amount of pressor material in the renal venous blood of 8 out of 11 patients with chronic heart failure. Schwartz et al (233) found that the renal renin content of cirrhotic patients who had died in an edematous state was significantly greater than normal. Blood circulating renin activity has been usually found elevated in patients, in edematous state such as, heart failure, cirrhosis and nephrotic syndrome. (234-237). Keley et al (238) published that the renin activity was significantly increased in the lymph of patients with congestive heart failure. In patients in such edematous states a hypergranularity of the JG cell has been reported (216, 239, 240). Serobrovskaya et al (241) have reported in cardiac and cirrhotic patients an increased JGC granularity as well as renal renin content. Bartter and co-workers (242, 243) have reported cases of hypokalemic alkalosis with secondary hyperaldosteronism with hypertrophy of the JG complex, increased granularity and elevated plasma renin activity. A similar case was reported by Shiffman et al (244), recently in our Institute (246). We have studied a patient from Doctors E.E. McGarry and J.C. Beck (Royal Victoria Hospital) which presented a similar clinical picture to those reported by Bartter et al. However, they found an increase of erythropoietin in the plasma and urine of the patient (245). The plasma renin activity values were also highly elevated, and ~~the~~ studies of surgical biopsy revealed a high JGI and JGCC (246).

Furthermore, in aminonucleoside nephrotic rats (with not such a high degree of ascites as those reported by Tobian (228)) in which these two parameters were studied in the same animals, no difference of the JGI at the 12th day of the experiment was observed as compared to the control group

(246). However, a very slight increase in plasma renin activity was encountered (247).

vi) Comments. It should be considered that the large amount of experimental work that has been done in rats, related to blood renin-like activity concentration, has been performed with very crude methods as the cross-circulation technique in which the total pressor effect of all circulating pressor substances is measured, besides the plasma renin activity. Moreover, data obtained under quite different experimental conditions have been related to each other, although they have not been directly comparable. The present work was concerned in greater part to see whether or not direct correlations of the JG cells and plasma renin activity exists when determining them in the same animals and under the same experimental conditions. Furthermore, plasma renin activity was studied with a more precise methodology.

8 - METABOLIC PATHWAYS: HISTOCHEMICAL STUDIES.

It has been established in both man and experimental animals that the juxtaglomerular cells are related to the formation of extractable renin in the kidney. However, very little is known of the type of metabolic pathway operating in these cells, and relatively few histoenzymatic studies focus their attention on the JG cells.

a) Amino-oxidase. Mustakallio et al (248) using histochemical techniques reported that the amino-oxidase activity in the rat kidney is mainly localized at the vascular pole of the glomerulus "presumably corresponding to the JG cells". They also observed in renal tissue obtained from unilaterally nephrectomized rats rendered hypertensive by renal constriction or desoxycorticosterone, that the number of juxtaglomerular cell groups showed a diminution in amino-oxidase activity. Whether or not this

observation is related to the concentration of catecholamines at the glomerular vascular pole remains to be elucidated. However, recently de Champlain et al (249) have reported in nearly similar experimental conditions in the rat a decrease in catecholamine renal content. Similar findings with Goldblatt kidneys in dogs were previously reported by Wegmann et al (250).

b) Alpha-glycerophosphate dehydrogenase (GPD). Hess and Pearse (251) reported that the mitochondrial alpha-glycerophosphate dehydrogenase could be demonstrated at the vascular poles of the glomeruli (presumably in the JG and Lacis cells) of rats using a histochemical technique. This technique was based on the metal chelation of formazan (see below) employing a tetrazolium salt (MTT) as a hydrogen acceptor and cobalt chloride.

They also observed that the GPD in the vascular poles of the glomeruli increased both in ischaemic kidneys from renal hypertensive rats and in kidneys from adrenalectomized rats without corticoid substitution or salt addition. In contrast, a decrease in activity was noted both in unclamped kidneys from animals with renal-clip hypertension and in animals treated by DCA and a high sodium diet. The authors concluded that the activity of these enzyme changes runs parallel with the renal renin content (and possibly the JG cell granularity) in both experimental hypertension and adrenal insufficiency and they are of the opinion that this may be one of the major metabolic steps which govern the functional state of the juxtaglomerular cells.

Dyrda (252) has observed that the GPD was more active in the JG cells of the rabbit than in the rat and dog and was slightly active in man.

However, in dogs with clamped kidneys, Dyrda did not observe any appreciable changes in the ischaemic or in the contralateral kidney.

Recently Swales and Lewin (253) studying transplanted kidneys, in man and dog, associated with ischemia, have observed in some kidneys moderate activity of GPD in JG cells. In others, however, the activity disappears completely. Swales and Morgan (254) have reported a release of vasopressor material occurs when ischaemic kidneys are grafted into canine and human recipients.

i) Expressions of the GPD. By biochemical assay of tissue homogenates it has been shown that alpha-glycerophosphate dehydrogenase (GPD) has a significant role in the glycolysis of the flight muscle of insects (255-260) and that it was associated with the mitochondrial fraction (261). Emmart (262) using a fluorescent antibody technique has shown in rat cardiac muscle that the distribution of the enzyme is related to the distribution of mitochondria and the band of the fiber identified by an antimyosin staining. Chefurka (263) has proposed that in insects glycolysis is catalysed not by lactic dehydrogenase, as it is in mammalian muscle, but by an alternative pathway, in which GPD plays a vital role in the conversion of reduced diphosphopyridine nucleotide.

This enzyme was originally described in rat muscle by Von Euler et al (264) and isolated in crystalline form from rabbit muscle by Baranowski (265). Bücher et al (256) have also shown that in invertebrate muscle GPD may be present in relatively high concentrations with lactic dehydrogenase. Baranowski (266) has postulated that this enzyme in muscle may be present in two states, the labile phase being extra-mitochondrial while the particulate phase is intramitochondrial. Hess and Gehm (267), and Schmidt et al

(272) have demonstrated the presence of GPD in normal human blood plasma and affirm its presence in tissue extracts. This enzyme has been described by Hess (268) as a plasma enzyme common to the cytoplasm of all tissues but of "unknown clinical significance".

The GPD has been involved in the synthesis of neutral fat (269). Alpha-glycerophosphate dehydrogenase mediates the conversion of dehydroxyacetone phosphate, by glycolysis, to the active glycerol (alpha-glycerophosphate) which then combines with fatty acids to form neutral fat. A high activity of GPD in seal adipose tissue has been found by Fried et al (270). They suggested that it may represent a high rate of active glycerol for the synthesis of neutral fat, or possibly that the high GPD is an adaptation to oxygen deprivation associated with arterial constriction during diving (271). Thus, this enzyme functions similarly to lactate dehydrogenase in providing for a reoxidation of the reduced NAD arising during glycolysis with a concomitant production of reduced metabolite.

D. LACIS CELLS (PSEUDO-MEISSNERIAN CELLS)

Described initially by Goormaghtigh (7), they are located in the triangle formed by the two glomerular arterioles and the macula densa.

1 - LIGHT AND ELECTRON MICROSCOPIC DESCRIPTION

Light microscopic studies have pointed out that the lacis cells as compared to the granular cells are smaller. The nuclei are elongated or ovoid, they do not contain specific granules and their cytoplasm is frequently clear which differentiates them from the typical smooth muscles cells of the arteriolar wall (7, 60). Since these groups of cells are greatly agyrophilic, Goormaghtigh (7, 10, 12) thought that they resembled tactile corpuscules. However, Oberling and Hatt (13, 14) demonstrate in electron microscopic studies that they have no resemblance to nervous structures. Their

cytoplasm appears devoid of dense osmophilic membrane-bound granules characteristic of the granular cell, but they contain few to moderate numbers of mitochondria and a Golgi apparatus. The endoplasmic reticulum is less extensive than in the granular cells and the ribosomes are generally fewer giving the cytoplasm a less dense appearance. Sometimes they have small dense cytoplasmic bodies (57) and some fibrillar bundles have been observed (57, 14). However, these findings as well as the presence of micropinocytic vesicles occur rarely (66). The nuclei of the lacis cells as described above, are of relatively small size, elongated or ovoid in shape, and sometimes indented. The chromatin pattern appears to be less dense than that of the granular cells and shows less marginal accumulation near the nuclear envelope (14, 57, 60).

Latta and Maunsbach (57) claimed to observe transitional forms of lacis cells to granular cells. However, they did not show any electronmicrographs of such possible transitional forms.

The lacis cells have cytoplasmic projections, and between them a rich network of intercellular substance or matrix material with a density like that of basement membranes (14, 57, 60). No blood or lymphatic capillaries seem to exist in this cellular conjunctive network (60).

In lacis cells of human kidneys, complexes formed by regular crystalline material have been found associated with droplets or granules and irregular granule material (79, 80, 85). The significance of these complexes is not known but they seem to increase with age (79). These lipofuscin-like complexes have also been found in granular, in mesangial (78) and in smooth muscle cells of arteriolar walls in other human tissues (79).

Finegan (273) has reported the presence of alkaline phosphatase activity located possibly in the lacis cells of the rat near the macula densa. However, he did not observe this finding in 19 other species of mammal examined. Alpha glycerophosphate dehydrogenase activity has also been noted in these cells (251).

E. MACULA DENSA

1 - LIGHT MICROSCOPY

Where the distal tubule comes in contact with the corresponding glomerular vascular pole, the tubular epithelium takes a particular morphology, that has long been reported (274, 9). Peter (9) described an agglomeration of nuclei; later, all the formation represented by an elliptic well defined plaque was called the macula densa (8).

The macula densa is composed by narrow palisadic cells, relatively high. The nuclei, close to one another are frequently located at different levels (8, 275). Becher (276) even thought of an epithelium often pluristratified. This arrangement of the nuclei is very characteristic and is not found anywhere else in the urinary tubules. Furthermore, it has been observed that the nuclei of the macula densa are smaller than those of other regions of the intermediary segment (10, 277). This has been confirmed by the caryometric studies of Bücher and E. Zimmermann (275).

On the other hand, there are different opinions concerning the nuclear structure: Goormaghtigh (10) considered it as very chromatic, however, Okkels (278) as rather light. There is the same divergence concerning the position of the nuclei. The former author and Becher (276) often found them in an apical position, whereas Okkels (278) considers them to be located in the center

of the cells, Zimmermann (8), Edwards (277) and Becher (276) mention the presence of relatively voluminous nucleoli.

On perusing the literature concerning the cytoplasmic structure, one can easily perceive the confusion. Goormaghtigh (10) precisely describes "that the basal region is pale ; it does not contain either granules nor filaments; among the cells of this segment there is always at least one that contains a very distinct vacuole. The apical pole is darker because of an accumulation of fine granulations". However, Edwards has noted in mammalian kidneys, a neutrophilic or lightly basophilic agranular cytoplasm (277). But the problem is still more complicated by the distinction made by de Muylder (279-282) in dog and man, of two sorts of cells in the macula densa, called chromophobic and chromophilic. Whereas the chromophobic cells are cubic with a round nucleus and an unstained cytoplasm or containing only a few fine acidophilic granules, the chromophilic cells are high and narrow with an elongated nucleus, and reveal a cytoplasm deeply stained in red (after coloration with erythrosine-orange G) without any visible granules ; its superior pole is swollen, sometimes prominent in the tubular lumen, and its inferior pole, at first narrow becomes wider when it contacts the basal membrane. Becher (276) pointed out that the structural characterisitc of the macula densa cells may change, considering that it apparently depends on their temporary functional state.

In contrast to the rest of the distal tubule, the macula densa cell contains few mitochondria (10, 278) being of small size and round (283).

McManus (284, 285) has pointed out in the cat and rabbit that conversely to adjacent tubule cells, the Golgi apparatus in cells of the macula densa is located on the basal side of the nuclei. This observation

was confirmed by Okkels (278). However, Fischer (285) reported that in tubular segments of mice kidneys, the cell structure as well as the localization of the Golgi apparatus may change in relation to the functional state of the cells. Furthermore, McManus (30) reported an absence of lipids in the mouse macula densa, in contrast to other parts of the tubular cells. Goormaghtigh (287) pointed out that the macula densa is a more resistant structure to kidney degenerative processes. Furthermore, some authors (30, 216, 288) have reported the possible discontinuity of the basement membrane of the macula densa.

2 - ELECTRON MICROSCOPY

With fine morphological studies further distinguishing features become more apparent (14, 57, 56, 66, 289, 290). The nuclei are sometimes more irregular than those in other cells of the distal tubule. The mitochondria are short and irregularly distributed in contrast to the characteristic palisading mitochondria of the adjacent portion of the distal tubule. The infolding basal membranes and interdigitating basal processes are fewer and much more irregularly arranged than other parts of the distal tubule. The plasma membranes at the base separate, sometimes forming extra-cellular or sub-basilar compartments. The Golgi apparatus is usually found in the basal part of the cell. The endoplasmic reticulum and the free ribosomes do not present any special characteristics. The basement membrane may be irregularly folded under the macula densa.

However, no electron microscopic studies have been reported presenting evidence of interrupted zones in the basal membrane of the macula densa.

It has also been pointed out that rarely the lumen of the tubule may separate adjacent cells and penetrate close to the base of the cell (57, 66). The luminal surface of the cells has a number of microvilli, and the plasma membrane seems to be thicker (67, 66).

Therefore, it has been emphasized by most authors (14, 289, 60, 66) that the macula densa cells represent only a trivial epithelium without sensory, or secretory characteristics.

Few electron microscopic works have reported studies about the macula densa under experimental conditions. Barajas and Latta (16) have shown relatively large amounts of small dark bodies in macula densa cells of adrenalectomized rats. They also observed that in some cases the macula densa seems to have a dilated endoplasmic reticulum and possibly dilated Golgi vesicles. Hatt (66) has shown dilated intercellular spaces in the macula densa of rats under high sodium intake.

3 - HISTOCHEMICAL STUDIES. HEXOSE MONOPHOSPHATE SHUNT.

a) General methodological considerations. Progress has been made in identifying trace metals required for the activity of a number of enzymes (291).

Nachlas et al have pointed out using histochemical procedures the activating effect of Ba^{++} on glucose-6-phosphate dehydrogenase, when (2, 2,-di-p-nitrophenyl-5,5'-diphenyl-3,3'-dimethoxy-4'biphenylene) ditetrazolium chloride (Nitro-BT) was used as a final electron acceptor (292)

Pearse (293, 294), described that when thiazolyl tetrazolium salts are used as electron acceptors in dehydrogenase reactions in the absence of a cheletor (Co^{++}, Ni^{++}, Cu^{++}) the formazan deposits rapidly aggregate and crystallize. In the presence of suitable metallic ions, on the other hand,

the deposits are non-crystalline. Thus a capture reaction has been established which prevents crystallization and which presumably also prevents diffusion of the free formazan. This observation forms the basis of the monotetrazolium-metal chelation methods of a variety of dehydrogenases and diaphorases (295, 296).

On the basis of the histochemical methods involving metal chelation of formazans, Hess et al (295) have reported using glucose-6-phosphate disodium salts or calcium salts as substrate, with CoCl2 and MTT (3-(4, 5-dimethylthiazolyl (-2)-2, 5-diphenyl tetrazolium bromide), that the glucose-6-phosphate dehydrogenase (G6PD) activity in rat kidneys is mainly located in the macula where it is highly active. The authors conclude that the intracellular localization of this enzyme is in the mitochondria since the formazan deposits were in the form of dots having an average diameter of 0.2 to 0.3μ confirming the previous finding of Scarpelli et al in various dehydrognases and diaphorases (295). A high specific activity of 6-phosphogluconic dehydrogenase in the macula cells of rat kidneys, was reported by Nachlas et al (292) using the barium salt of 6-phosphogluconic acid as substrate, and NBT as final electron acceptor, without adding a metal salt.

In contrast to the finding of Scarpelli et al (295) and Hess et al (296), Nachlas et al (292) observed that the intracellular localization of various dehydrogenases and diaphorases studied appeared as fine granules, never rods or filaments, often closely packed in the cytoplasm, and they suggested that these enzymes are associated with the microsomes. This point of view is the same as that reported by Farber et al (297), and Sternberg et al (298). Furthermore, biochemical studies on homogenates

in isotonic media by Glock and McLean (299) strongly suggested that the glucose-6-phosphate dehydrogenase is located in the microsomes. A similar location has been described by Dixon and Webb (300).

b) Expressions of the hexosemonophosphate shunt.

The hexosemonophosphate pathway comes under the guise of many aliases; notable among them are such names, as the phosphogluconate oxidation pathway, the oxidative pentose phosphate cycle, the Warburg-Lipmann-Dickens pathway the direct oxidative pathway (301, 302).

In brief, the operation of this cycle is as follows. A hexose molecule enters the pathway in the form of its monophosphate. The latter is oxidized by "glucose-6-phosphate dehydrogenase", to the lactone of phosphogluconic acid. This compound is then split by a lactonase. The resulting phosphogluconate is then decarboxylated by "6-phosphogluconic dehydrogenase", to a pentose phosphate. The product (ribulose phosphate) may be used for the synthesis of nucleotides and thus for the production of nucleic acids, or it may be carried through the pentose cycle. The pentose phosphate rearranges and condenses with another pentose phosphate to form a heptulose monophosphate and a triose phosphate. These two compounds eventually are reconverted to a hexose monophosphate and a tetrose phosphate. The pathway is shown in detail in fig. 2. It is noteworthy that reduced triphosphopyridine nucleotide (TPNH) is produced.

The pentose phosphate cycle serves three purposes: the furnishing of intermediates, reduced triphosphopyridine nucleotide and, perhaps energy. Triose phosphate may be used for carbohydrate synthesis as in the case of the carbon reduction cycle (303), tetrose phosphate for the synthesis of aromatic acids via shikimic acid (304), pentose phosphates for the synthesis

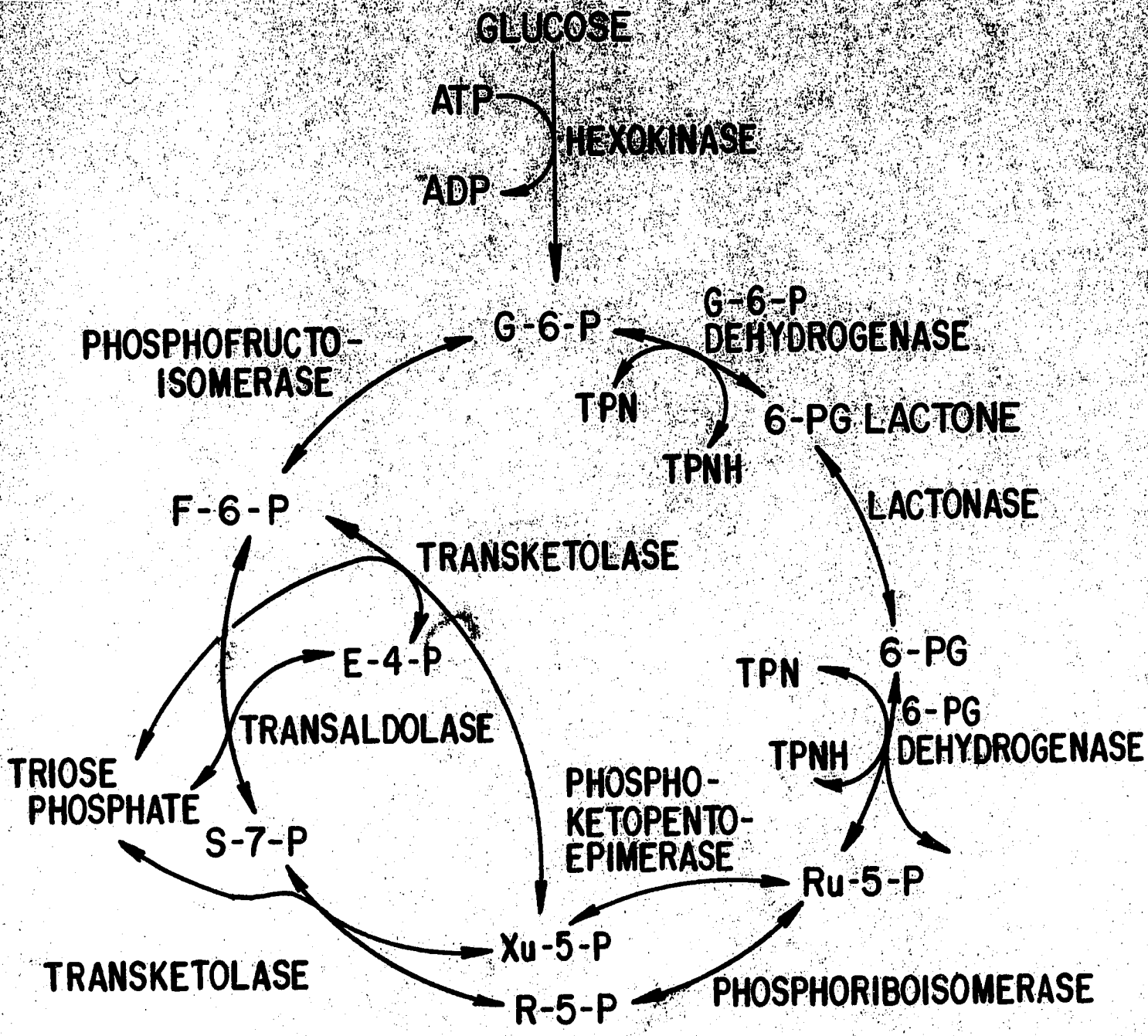


FIG 2. THE HEXOSE-MONOPHOSPHATE SHUNT (302)

of nucleic acids (305, 307).

As it has been indicated, one of the major requirements fulfilled by the hexosemonophosphate pathway is for the production of reduced triphosphopyridine nucleotide (TPNH). This reduced coenzyme functions in a number of important synthetic processes, it appears to be required for the reductive amination of alpha-ketoglutarate to glutarate, the reaction which serves as the primary source of amino nitrogen in the cell (308). In the biosynthesis of lipids, TPNH plays an important role. Two equivalents are required; one for the reduction of the ketoacyl-enzyme intermediate to the hydroxyacyl-enzyme intermediate and the second for the reduction of the double bond to form the saturated fatty acid intermediate (309). Flatt and Ball (310) have pointed out that approximately half of the TPNH required for fatty acid synthesis is derived from the hexosemonophosphate pathway, while the rest is generated by the malic enzyme. TPNH participates in several steps in the biosynthesis of steroids, including ring closure (311), hydroxylation (312) and reductive demethylation, (313) and is essential for aromatic hydroxylations in both the case of steroids and the biosynthesis of ascorbic acid. The reduction of glucuronic acid to gluconic acid requires TPNH (314). The reduction of ribonucleotides to deoxyribonucleotides has been shown to be a specific TPNH system (315) and finally in photosynthesis TPNH generated by the photosynthetic apparatus is utilized for the reduction of 3-phosphoglycerate, a key step in photosynthesis (316).

As an energy source, the hexosemonophosphate pathway may play a lesser role than glycolysis as so far there is no evidence for the direct coupling of TPNH with oxidative phosphorylation (317), although, TPNH can transfer its

hydrogen to diphosphopyridine nucleotide (DPN^+) via the transhydrogenase reaction. Other means, however are possible for the formation of ATP : (a) substrate level phosphorylation of adenosine diphosphate during the oxidation of the triose phosphate ; and (b) phosphoroclastic cleavage of either fructose-6-phosphate or xylulose-5-phosphate to form acetyl phosphate (318).

Recently, Horecker (308) has suggested another function for the hexosemonophosphate pathway, to remove the accumulation of glucose-6-phosphate. Glucose-6-phosphate has been shown to inhibit the phosphorylation of hexose by hexokinase (319) and thus prevents the entry of D glucose into the metabolic pathway, while at the same time it promotes the conversion of glucose to glycogen (320). The role of glucose-6-phosphate in the control uptake of hexose has been elegantly illustrated in the case of the red cell. Rose and O'Connell (321) have demonstrated that the rate of uptake of glucose by the red cells is directly related to the level of glucose-6-phosphate in the cell. They were able to modify the steady state level of glucose-6-phosphate in two ways. It could be increased by adding a precursor or glucose-6-phosphate, such as inosine, which gives rise to pentose phosphate and immediately to glucose phosphate. It could be decreased by adding methylene blue, which catalyzes the oxidation of TPNH by oxygen and accelerates the removal of glucose-6-phosphate.

c) Macula densa enzymatic activity in various experimental conditions.

Hess and Pearse (322) described, in rats rendered hypertensive by unilateral clamping of the renal artery, that a high activity of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase occurs in the macula densa of the clamped kidney. In contrast, this activity was

markedly decreased in the macular segment of the contralateral untouched kidney. These authors also observed that excision of the clamped kidney in chronically hypertensive rats resulting in the maintenance of elevated blood pressure, was followed by an increase of the activity in the macula densa of the remaining kidney. They suggested that this enzyme system in the macula densa is implicated in the formation of a renal pressor principle. As described earlier, Hess and Regoli (157) following the chronology in a relatively reduced number of clip-hypertensive rats, have claimed in the early ischaemic period (animals 2 days after applying the clamp) that the first changes were the increased activity of the glucose-6-phosphate dehydrogenase (G6PD) in the macula densa accompanied by a rise in renal renin content. In spite of this fact the JGI did not change significantly. Furthermore, after 5 days the clamped kidneys showed an increase in the granulation and enzymatic activity (alpha-glycerophosphate dehydrogenase), accompanied by an increase of G6PD activity in the macula densa and a rise in renal renin content. They suggested (also based on the work of Bing and Kazimierczak (156) who found both renin and G6PD in the premacula densa of immature pig nephrons), that renin (or a precursor) is transferred continuously from the macula densa to the granulated JG cells. The latter, functioning as moderators of renin storage and release into the circulation, might change their appearance in response to an altered output of the macula densa. However, after 7 days they observed in the three studied parameters a parallel decrease in the untouched kidney.

Hess and Gross (323) noted a decrease in G6PD activity in the macula densa as well as renin in the kidneys of rats with hypertension induced by the administration of desoxycorticosterone and salt. Inversely a marked increase in G6PD activity of the macula densa was obtained in adrenalectomized animals together with a low salt diet. However, control animals kept on a low sodium diet alone for 7 days, was followed by a slight increase of activity but the change was not considered significant. As described earlier, Gross and Sulser (133) have found an increase of renal renin content in rats with adrenal insufficiency.

Fisher (324) has found a close correlation between the degree of juxtaglomerular granulation, hexosemonophosphate shunt enzymes of the macula densa and pressor (renin) activities in the kidneys of rats with a variety of forms of experimental hypertension (unilateral renal artery constriction, ureteral ligation, figure 8-compression, DCA + NaCl). The experiment was terminated at the beginning of the 4th week. However, in the parameter studied, a relatively large percentage of animals gave values, in the manipulated kidney, very close to the intact animals. Morever, in fig. 8-compression the values that he reported were very close to the control group. (140)

Dyrda (252) has pointed out that various normal species differ in G6PD and 6PGD activity of the macular segments. This author also reported that in dog Goldblatt kidneys, the G6PD activity in the macula densa of the clamped kidneys was as in the control animal. However, this activity was decreased in the unclamped kidney. On the other hand,

in kidney specimens from hypertensive patients, he did not observe significant changes in the hexose-monophosphate shunt enzymes of the macula densa.

Swales and Lewin (253) have reported in human and dog ischaemic kidneys a moderate activity of the enzymes of the macula densa in some cases. However in other cases, the activity was greatly diminished.

It has been reported that no significant changes occur in the G6PD activity of the macula densa in aminonucleoside nephrotic rats (325) and in rats after acute bleeding (157).

Marx and Dean (326) have pointed out that weanling rats receiving a low-sodium, high potassium diet, showed progressive kidney and adrenal changes over an 11-week period. The JGI increased with time. Thus after 2 weeks, they observed a significant increase of the JGI. G6PD and 6PGD activity in the macula densa was observed to increase after 4 weeks and this activity became more marked in animals deficient for a longer time.

Gross and Hess (327) have reported that the G6PD activity in salivary duct epithelium of rats is decreased simultaneously to the macula densa of the unclamped kidney in the clip-hypertensive as well as in the macula densa of the metacorticoid hypertensive. These findings led the investigators to the following conclusions: "The kidney, as well as the salivary glands are secretory organs eliminating salt and water, and similar changes in enzymatic activity in their duct system may be the expression of attempts to adjust the function of these organs to special conditions (sodium retention) that are presenting hypertension".

Recently, Capelli et al (328) have confirmed the finding of Gross

and Hess (327) in metacorticoid hypertension. Furthermore, when an animal with DCA and NaCl received angiotensin II, they observed an increased G6PD activity in the first half of the proximal tubules, adrenal and salivary glands, with a marked decrease in renal renin content. In contrast, in a rat kept on a sodium deficient diet, they observed an increased G6PD activity in proximal tubules, macula densa, adrenal zona glomerulosa, and salivary gland, accompanied by an increase in JGI and renal renin content. The authors concluded that G6PD appears to be of importance in the interaction of angiotensin and sodium balance; that the macula densa appears sensitive to changes in Na metabolism; and that angiotensin affects proximal tubular enzyme activity.

Evidence for an important role of the hexosemonophosphate shunt in renal reabsorption processes is relatively indirect. In a histochemical study, Himmelhoch et al (329) produced an inhibition of the G6PD activity in the proximal tubule of the necturus by adding ouabain to the incubation medium, thus implicating the hexose monophosphate shunt in the cellular transport of sodium. Later, Karnovsky and Himmelhoch (330) found that in the proximal tubules of frog kidneys, the enzymes of both glycolysis and the hexose-monophosphate shunt were present in very high activity. They suggested a possible role of these enzymes in proximal tubular transport processes.

In a recent biochemical study, Dies and Lotspeich (331) reported that the activity of the hexose monophosphate (HMP) shunt dehydrogenases, measured in rat kidney homogenates, was markedly increased in animals with NH_4Cl acidosis. Sodium depletion also enhanced the activity of HMP

shunt's enzymes. They also found a significant correlation between HMP enzymes and acid excretion. These investigators suggest a possible role of the HMP shunt dehydrogenases in acid excretion.

F. EMBRYOLOGY OF THE JG COMPLEX

Various groups of authors (332-334, 337, 338) indicate that the renal corpuscule develops in situ (Herring theory (336) from undifferenciated cell groups and not by invagination of capillaries of the blind end of a previously formed tubule (Bowman theory (335). Recent information on what may happen in the development of the juxtaglomerular apparatus in rats, piglets (338) and humans (332-334), seems to indicate that endothelial, mesangial, lacis, and juxtaglomerular cells have the same origin from the mesenchymal flocculus; this finding supports the concept that juxtaglomerular and lacis cells are continuous with the mesangial cells, and that both can be transformed or differentiated in granular cells (14, 339-341). A part of the internal primordial epithelium that will form a layer of the tubular epithelium at the level of the distal tubule nearest the vascular pole, differentiates to become the macula densa. At this developmental period, fenestration of the basal membrane of the macula densa has been noted (338).

There are in the literature some controversial findings concerning which period of kidney development the granules appear in the vascular pole of the glomeruli. Thus juxtaglomerular granulated cells have been identified in fish and amphibia possessing a persistent mesonephros in adult life (6, 277, 281, 40, 161). In the mesonephros of hog embryo Kaplan and Friedman (342) have suggested that the juxtaglomerular cells

were absent. On the other hand, Sutherland and Hartroft (343), have identified juxtaglomerular granulated cells and the macula densa in the mesonephros and metanephros of hog embryo. Furthermore, the presence of the macula densa and afferent arteriole has been found in the new born piglet's kidney (338). Macula densa has been observed in the rabbit mesonephros (281) and, in mesonephros and metanephros (281) of human embryos. Epithelioid cells in the glomerular arterioles have been also identified in the mesonephros and metanephros of human embryos (281, 344).

Ruyter (1) has described in the post-natal mouse's kidney that juxtaglomerular granular cells appear about one week after birth and they are well differentiated after two or three weeks. Moreover, Friedberg (345) has observed juxtaglomerular granules soon after birth in the renal cortex of mice and initially they appear in greatest concentration in the juxtamedullary zone of the kidney. However, within three weeks this pattern of distribution is reversed, and at adulthood, a higher concentration of granules is present in the subcapsular cortex than in the juxtamedullary zone. Dauda and Endes (346) found that in the rat the granulated cells cannot be demonstrated before the fourteenth day of extrauterine life. Boss (338) has described in rats that the differentiation of the macula densa begins to occur on the twenty third day after birth.

There is a lack of information on the rate at which these granules develop in the new-born. In children and infants (151), the juxtaglomerular granules are fewer compared to those in adults. Recently, Dunihue et al (347) have noted an involution of the juxtaglomerular granulated cells in old rats.

G - COMPARATIVE MORPHOLOGY OF THE JG COMPLEX

Light microscopic studies have shown the presence of the juxtaglomerular complex in many species including man. On the phylogenetic scale (281), the juxtaglomerular cells first appear in the telecosts fish, but neither lacis cells nor the macula densa were observed (40, 161, 281). This similarly occurs in some amphibia "urodeles" (281) and reptiles (277, 281). In other amphibian species such as "anura", the juxtaglomerular cells and macula densa appear together (5, 6, 281, 343). In birds, contrary to lacis cells the juxtaglomerular cells and macula densa are well developed (277, 281, 348). In all mammals studied the macula densa, juxtaglomerular, and lacis cells have been found, although the granularity of the JG cells and the height of the macula densa vary in different species (1, 3, 8-10, 275, 281, 349).

Electron microscopic studies on the vascular pole of the glomerulus have been performed in a number of species including, fish (161, 350), amphibia (351), mouse (352, 289, 68), rat (14, 56, 57, 65), rabbit (65, 84), dog (10, 56), monkey (77), and man (79, 80, 85, 86). Juxtaglomerular cells are similar in all of them. Species differences in the electron density and structure of the granules have been noted. In fish (350), different crystalline-like granular structures have been observed. In the human and monkey the granules are denser than those of the rat, (78). The proto-granules of the former two have a polygonal shape, while those of the rat tend to be more ovoid (78).

Different degrees in granularity have been found depending on the topographical location and the studied nephron. It has been shown in adult

rats (353) that there are a higher number of juxtaglomerular granules and epithelioid cells in the afferent vessels of the subcapsular (short nephrons) as compared to the juxtamedullary nephrons (long nephrons). The same distribution has been confirmed in mice (48, 49), rabbits (160, 354), human and cats (355). A correlation with renin distribution has been demonstrated in rabbits (159) and humans (355). Friedberg (48) has suggested that such differences between the cortex and the juxtamedullary regions might well depend of the length of Henle's loops.

Topographical studies (214) on the renal cortex in rats have shown that the JGI of granularity is higher in the greater curvature than in the perihilar cortex,but despite this fact, Friedberg (48) has described in mouse an equal distribution of the macula densa in all zones of the cortex. Faarup (353) has noted in rats that the number of macula densa cells in the subcapsular cortex is higher compared to those of the juxtamedullary zone and inversely, the lacis cells were more increased in the latter zone than those in the subcapsular region.

Rapp (356) has presented evidence that by selective imbreeding in mice with a high and low juxtaglomerular index of granularity for four generations, it is possible to obtain selective lines with high or low JGI of granularity. Recently Rapp (357) has reported that following unilateral nephrectomy, kidney weight increased significantly, more with saline treatment in mice bred for a low JGI than those bred for a high JGI. Therefore he suggested that such variations might reflect differences in renal handling of sodium.

H - RELATIONSHIP BETWEEN JUXTAGLOMERULAR COMPONENTS AND RELATED STRUCTURES.

An intimate and constant relationship between the distal tubule and the glomerular arterioles has been established by light microscopy, (274, 8, 10, 12) and electron microscopy (14, 56, 57). Barajas and Latta (290) and Faarup (353) studied this relationship in three dimensional models. They noted that the ascending limb of the loop of Henle rises from the medulla, contacts the efferent arteriole first and runs along it toward the hilus of the glomerulus, contacts the afferent arteriole and then turns into the convoluted portion of the distal tubule. Barajas and Latta (290) according to a previous observation by de Castro and de LaPeña (288) suggested that the contact between the macula densa with the efferent arteriole was more common than with the afferent arteriole. Faarup found that the contact between the macula densa and both the afferent and the efferent vessels were variable. However, contact with the afferent vessels was slightly more common than with efferent vessels. (353)

Electron microscopic studies have pointed out that the macula densa was separated from the arteriolar wall by a basement membrane at the site of contact (14, 60, 66), but projections of macula densa extending into the arteriolar walls have been described (14, 57, 290).

A basement membrane-like matrix also separates the cells of the non-tubular component of the juxtaglomerular complex from each other (14, 60, 66).

Electron microscopic observations give support to suggestions in earlier literature based on light microscopy that JG cells are modified smooth muscle cells. Although smooth muscle cells of the glomerular

arterioles are typical of vascular smooth muscle cells found in arterioles throughout the body, they are apparently unique in that they may be replaced by JG cells. This transformation has been suggested by several authors (1, 14,56, 84) due to the presence of bundles of fibrils or myofilaments in granular JG cells.

Although granular cells may sometimes be found in the wall of the efferent arteriole (16, 50, 53), they are much more prominent and generally present in the afferent arteriole.

Continuity between JG complex and the mesangium of the glomerulus was suggested by the work of Zimmerman (8) and is further indicated by electron microscopy (14, 339, 341, 16). The mesangial cells have much in common with the lacis cells, the afferent and efferent arteriole. They seem to have the same embryological origin (332-334). The mesangial cells also contain some fibrillar bundles (14, 57). Goormaghtigh (12) had found appreciable numbers of granulated cells in the mesangial region of rabbits with constricted renal arteries. Dunihue and Boldosser (340) demonstrated large numbers of granules in the mesangial cells of cats 2 years after adrenalectomy. In contrast, Barajas & Latta (16) have found little, if any, increase in the number of granular cells of the mesangium of adrenalectomized rats after 75 days by either light or electron microscopy.

Another similarity is that the Lacis cells and to a less extent the JG cells can phagocytize intravenously injected thorotrast, although the amount is much less than that taken up by mesangial cells (340, 341).

Finally, it has been suggested that in some experimental conditions [illegible] the lacis cell may become granulated cells (57, 66, 84). Reeves & al (239) claimed that in cirrhotic patients, basal hydropic changes in the macula densa are correlated with an increased number of JG cells, mostly with the type IV of the Turgeon and Sommers classification (large agranular cells with a clear, watery cytoplasm).

I - INNERVATION OF THE JG COMPLEX

1. LIGHT MICROSCOPY.

Light microscopists describe a rich innervation of the JG complex, (1, 3, 127, 279, 282, 358, 359) by a silver impregnation method, Hartman and Davies (360) described postganglionic fibers with afferent endings at frequent intervals in the adventitia of smaller renal arteries. They also described endings, assumed to be afferent and possibly sensory among JG cells in the afferent arteriole of cats and rats. Christensen et al (361) corroborated the presence of nerve fiber terminations in juxtaglomerular "bodies" of cats but declined to classify them into efferent or afferent categories. De Castro and de la Peña (288) observed a rich sympathetic innervation in both afferent and efferent glomerular arterioles of human kidneys. They also observed nerves around Bowman's capsule which they assumed to be of an afferent viscero-sensory nature. Fourman (362) described the efferent arterioles of the deep glomeruli as having an abundant nerve supply which is absent in the superficial glomeruli. Recently, by application of a sensitive fluorescent method, Nilsson (363) characterized the nerve terminals surrounding the small arteries of the kidney, including the afferent arteriole, as adrenergic, thus providing convincing evidence for the previously assumed sympathetic

innervation of this structure. He also found that this adrenergic innervation ended at the glomeruli and was not associated with the capsule, with veins or tubules.

2 - ELECTRON MICROSCOPY.

Barajas (364) was the first to study the innervation of the JG complex by electron microscopy. He found numerous non-myelinated nerves in association with the arteriolar walls in the monkey JG complex. In the proximity of smooth muscle cells or granular JG cells of the arteriolar walls the nerve fibers acquire the appearance of vesiculated processes. They appear to be separated from the smooth muscle plasma membrane by two basement membranes, one belonging to the arteriolar wall and the other to the axon-Schwann cell complex, with a gap of 1000 to 2000 A°. He also described in the monkey, dark and light vesiculated processes, depending upon the concentration of vesicles and density of the cytoplasm. They appeared to be part of the same axon-Schwann cell complex with possibly some relation. Barajas also pointed out that in the rat the nerve elements were less numerous and a distinction between the two types of vesiculated processes was not apparent. This species difference in frequency of nerve elements seen by electron microscopy was also noted in studies by Hartroft (365). However, his electron micrographs do not show in rats details concerning with granular JG cells. Barajas (364) emphasized that the vesicular processes may well represent the sympathetic synaptic end nerve since they present characteristics, as granular vesicles, as those described in the adrenergic type. Barajas and Hartroft (366) described in

various mammals studied that the closer approach of the nerves to arteriolar smooth muscle cells or JG cells, was separated by an interval of approximately 1000 A^{o}. However, in the frog, membrane-to-membrane contact of nerve elements with projections of JG cells was observed by Hartroft (365).

Fisher et al (70) in one electron micrograph showed an axon-Schwann cell complex (without characteristics of a nerve ending) associated with the JG complex and he claimed that few nerve elements are related to the JG cells in the human.

Hinglais et al (86) in one electron micrograph showed an axon-Schwann cell complex associated with a Lacis cell in the JG complex of the human being.

A further observation made by Barajas and Hartroft (366) was the presence of a nerve ending in close contact with the macula densa. These elements were also adrenergic in type. They were located either between the macula densa and JG cells, where they were separated by a small amount of basement membrane material or in an infolding of the macula densa where they were in still closer contact. However, the plane of section in their pictures should be considered and the possibility that those nerve endings may well represent presynaptic dilatations approaching the arteriolar wall.

J - ROLE OF THE JG COMPLEX.

From the foregoing review, the function of the JG complex may be equated to that of the renin-angiotensin system. This is a dual function, first in renal sodium and water regulation, and, secondly, by its pressor activity, in blood pressure regulation.

1. RENAL SODIUM AND WATER REGULATION.

a) Direct renal action.

Angiotensin has a diuretic and natriuretic effect. The result varies with the animal species studied. It is natriuretic in the rat (367), chicken (368), rabbit (369, 370) and cat (371, 372). However, it has antinatriuretic effects in nornal dog (373) and man (121, 374-76).

i) Regulation of the proximal intratubular hydrostatic pressure.

Leyssac (377) found, based on micropuncture studies in rats, that angiotensin causes a depression of the proximal tubular reabsorption rate. Also, he made the hypothesis that any reduction in filtration pressure (e.g. reflex spasm of the afferent arteriole, obstruction of the renal artery etc.) or any reduction in proximal intratubular pressure induced a primary increase of the release of angiotensin, by some chemical, mechanical or electrical change at the site of the macula densa resulting from the minor decrease in proximal intratubular pressure, consequently, the rate of proximal reabsorption is depressed and thereby lowers glomerular filtration rate. The constriction of the efferent arteriole and the depressed rate of reabsorption tend to maintain the proximal intratubular pressure constant.

Leyssac concluded that the physiological function of the JG complex is to serve as a feed-back mechanism whereby a change of the proximal intratubular pressure (i.e. the output of the regulated system) at the site of macula densa and via the release of angiotensin changes the proximal salt reabsorption and adjusts the intrarenal vascular resistance in such a way that the proximal intratubular hydrostatic pressure is maintained.

ii) Autoregulation of the renal blood flow.

Thurau's (378-81) group, however, emphasizes that besides its vasoconstrictor action, angiotensin has no direct effect upon tubular sodium reabsorption. He postulated that the possible physiological role of the JG complex is the "autoregulation of the renal blood flow", by the existence of a specific sodium feed-back mechanism between tubular and vascular structures at the level of the JG complex, which acts inversely between sodium concentration in the tubular fluid at the macula densa cells and glomerular filtration rate (GFR). He supports this hypothesis by the following experimental findings. An increase in Na^+ in one macula densa segment, locally induced by microinjection, reduced GFR of the individual nephron only, possibly by the locally formed angiotensin and subsequent pre-glomerular vasoconstriction, since the sensitivity depends on the renin concentration in the renal tissue. Furthermore, the low renin content of JG complex in juxtamedullary position is consistent with the observation that medullary blood flow is not, or far less, autoregulated than cortical blood flow.

b) Indirect action.

The JG complex activity is closely related to the zona glomerulosa of the adrenals and aldosterone secretion.

Dean and Masson (382) reported that renin injections cause changes in the width of the zona glomerulosa of the adrenals, a finding later confirmed by Kuhn et al (146).

Hartroft and Hartroft (384) demonstrated in sodium deficient rats and in human diseases with a low serum sodium a correlation between the degree of granularity of the JG cells and the width of the zona glomeru-

losa of the adrenals.

Since angiotensin stimulates the suprarenal gland in man to produce aldosterone as discovered by Genest et al (121) and later confirmed by Laragh et al (383), the physiological and pathological relation between the suprarenal and the kidneys have been strengthened. Perfusion of angiotensin in sheep (385), and dog (386, 387) stimulated the secretion of aldosterone. These effects are controversial in the rat (388, 389). However, chronic administration of angiotensin in rat produced a hypertrophy of the zona glomerulosa of the adrenal gland (65, 390, 39) with histochemical evidence of increased secretory activity (391).

In experimental and pathological conditions a relationship of JG cell granularity and hypertrophy of zona glomerulosa, or an increase in aldosterone, or both, occur as a result of renal ischaemia, especially the ischaemic kidney in unilateral renal hypertension (65, 392-394), ascites (227, 228, 53, 216) cases of hyperaldosteronism with hypokalemic alkalosis, but no hypertension (242, 244, 395, 396) and other conditions of secondary hyper-aldosteronism (397).

2 - BLOOD PRESSURE REGULATION:

a) Physiological role.

A recent contribution indicates a physiologic function of the renin angiotensin system in upright postural homeostasis. A significant increase in plasma renin activity has been reported in normal subjects in standing position as compared to normal subjects who remained in a reclining position (398). Slaton and Biglieri (399) have suggested a physiologic role in the Valsava experiment. The role of angiotensin in the basal pressure is rela-

tively hypothetical since the injection of hog antirenin to dogs does not change their blood pressure (595). Furthermore, angiotensin has a cardiovascular action most likely mediated by the sympathetic nervous system (225, 400-405).

b) Experimental renal hypertension

The role of the JG complex ceteris paribus renin-angiotensin in renal hypertension has been a debated problem and still there is too much conflicting evidence in both the laboratory animal and man. However, some lines of investigation, mostly related to ischaemic kidneys, suggest that in some, but not in all, experimental or pathological situations, the JG complex might play a role in the acute phase of the development of hypertension.

In 1937, Houssay and Fasciolo (406), based on the work of Goldblatt et al, (407) were the first to point out that renal hypertension was mediated through a humoral mechanism. After grafting ischaemic kidneys into the neck of nephrectomized dogs, they observed a rise in blood pressure.

During the acute phase of development of hypertension after renal blood restriction, a hyperplasia and hypergranularity of the ischaemic kidney have been frequently observed while the opposite kidney shows an almost complete absence of JG cell granules (11, 27, 138, 140, 223). In acute situations an increase in renin content of the ischemic kidney, and a decrease in the contralateral elevated plasma renin activity (413-417) and blood angiotensin have also been reported (418-420, 140, 409-412). When the hypertension becomes chronically established after several weeks or months, the granularity of the ischaemic kidney decreases in many instances (129,65)

and the renal renin content (415-417, 420) and angiotensin (423, 424) decreases also. On the other hand, various investigators have not found any correlation between the level of blood pressure and the JG cell granularity (65, 138, 140, 223, 425) or the renal renin content. Along this line of evidence, Regoli et al (222, 426) reported that if the unclamped kidney is excised in renal-clip hypertensive rats, the level of blood pressure remains unchanged despite a progressive decrease in renin content to normal values within three weeks in the remaining clamped kidney. Moreover, when unilateral nephrectomy is performed before clamping the remaining kidney, the same degree of hypertension develops within the same time as in non-nephrectomized rats, but no change can be detected in the renin content of the ischaemic kidney. Gross's group (198, 204) further demonstrated that a normal concentration of renin-like material is circulating in unilateral nephrectomized renal-clip hypertensive rats while it is greatly increased in non nephrectomized hypertensive animals. The same group (198, 204) suggested that the development of hypertension due to the renal artery clip does not require renin. Heptinstall (425) reported that in unilateral nephrectomized renal-clip hypertension, after 2 weeks, the JGI remains unchanged as compared to the JGI of the removed kidney. Moreover, an increase in JGI occurs in the clamped kidney when the remaining kidney is not removed.

Bing (427) attempted to determine whether renal hypertension could be developed in rats treated with DCA and salt to deplete the kidneys of renin. Some of the rats did not develop hypertension after three weeks on DCA and salt and this group had a clip applied to their renal artery.

Despite the fact that the renin content of the kidney was only 2 to 4% of the normal, the rise in blood pressure was similar to that obtained in normal rats with a clip applied to the artery.

From the foregoing review the renin-angiotensin system in experimental renal hypertension appears to play an initiative role in the development of hypertension in some experimental situations. However, in others it is very difficult to explain its possible role. On the other hand in chronic situations, its role is doubtful.

c) Human hypertension associated with renal diseases. Its clinical significance.

The role played by the JG complex in hypertension associated with renal disease is highly controversial. For some authors a hyperactivity of the JG cells was found accompanying various renal diseases (45, 216, 428-437, 393). In contrast some other authors reported no significant changes or decreased morphological activity (127, 281, 427-439).

However, in recent years a clinico-pathological significance of the JG complex has been emphasized in order to select patients with renal diseases associated with hypertension, which could be cured or improved by nephrectomy or vascular reparative surgery.

Turgeon and Sommers (73) reported that in renal ischaemia producing hypertension, patients with marked juxtaglomerular hyperplasia or granularity responded well to nephrectomy. Similar findings have been reported by Crocker et al (393) and Itskowitz et al (435). Further, Boughton and Sommers (436) have described hyperplasia of the JG body in a variety of unilateral

renal lesions that have caused hypertension and improved following nephrectomy. These included renal artery stenosis, hydronephrosis, chronic pyelonephritis, perirenal fibrosis and hypernephroma. In the kidneys with pyelonephritis the hyperplasia was present in the non infected areas. In hypertension associated with renal artery obstruction, our Institute (141, 142) considers, besides the morphological changes of the JG complex, three other parameters 1) the pre and poststenotic systolic pressure gradient, 2) renin activity in peripheral or in renal venous blood and 3) the presence or absence of the arteriolar lesions in the involved kidney. Considering these parameters, it was reported that patients suffering from hypertension associated with renal artery obstruction, having a significant systolic pressure gradient, a high blood renin activity and/or JG cell hypergranularity accompanied by relative hyperplasia, and absence of the arteriolar lesion in the involved kidney, were cured or significantly improved after the nephrectomy or vascular reparative surgery.

Finally, the importance of determining renin activity in patients with renal artery obstruction with biochemical and clinical features of primary aldosteronism has been established by Kirkendall et al (443), Conn et al (446), DeChamplain et al (444), Brown et al (447) following demonstration of undetectable or minimal arterial angiotensin levels in cases of primary aldosteronism secondary to adrenal adenoma (441, 442). Following the suggestion made by Genest (445), Kirdendal et al (443), Laragh et al (448) and Conn (448), that the measurement of renin activity might well prove of value in the differential diagnosis of this condition versus that

due to renal artery stenosis. Moereover, a disappearance of the JG granules and a relative hypoplasia occur in such cases of primary aldosteronism (394, 435, 451, 452).

K - NATURE OF THE STIMULUS ACTING ON THE JG COMPLEX

Factors that affect secretory activity of JG cells, as reflected by cytological changes and renin activity, are actually a debated problem and it has not been completely solved. Several theories have been proposed.

1) HEMODYNAMIC CHANGES

a) Changes in renal perfusion pressure: "stretch receptor" theory. Tobian (137, 203) has suggested that changes in mean renal arterial pressure might be the major factor controlling the release of renin by the JG cells. While perfusing isolated kidneys at various pressures, they observed, when perfusing with a high pressure, the JG cells granularity fell significantly, whereas no change was observed when the kidney was perfused at normal mean pressure. They postulated that, by their situation in the wall of afferent arterioles, the JG cells would act as a baroreceptor or stretch-receptor. A decrease in perfusion pressure would result in an increased secretion of renin which would restore and maintain an adequate perfusion pressure, while an increased perfusion pressure inhibit the production of renin. Skinner et al (225) gave support to this hypothesis when they demonstrated that the stimulus for renin release is primarily a reduction of mean inflow pressure, rather than a reduction in pulse pressure or total renal blood flow, Koletsky and Rivera Velez (454) have also reported that a rise of inflow pressure diminishes the level of circulating renin.

Tobian (137, 203) claimed on the basis of "the stretch receptor" theory that certain experimental manoeuvres cause hypergranularity of the JG cells and others which cause a diminution in granularity may fit well with this hypothesis. Taquini et al (202) found no change in the renal renin content of animals in shock for 30 minutes. In unilateral nephrectomized rats, the renin content of the kidney and the release of renin do not increase after clamping the remaining kidney. Nolla-Panades and Simpson (453) have reported that no significant change occur in JG cell granularity following clamping of the aorta above both renal arteries. In rat kidneys with genetic hypertension the results are contradictory, Smirk, and Phelan (456) , found no change in JG cells as compared to normotensive rats. However, Sokabe (455) found that a hypertensive strain of rats has about half as much renin in the kidney cortical tissue as a normotensive strain of rats. He also reported that the JG cell granularity was decreased.

b) Changes in intravascular volume:

Acute changes in blood and fluid volume have an effect on renin release without significant changes in JG cells granularity. Acute severe hemorrhage in rats quickly raises the level of circulating renin (148) without significant changes in renal renin content (198) or JGI (200). Moreover, hypertransfusion in rats which raises the blood volume has the effect of decreasing the level of circulating renin within 24 hours, without significant changes in renal renin content (198). Our Institute (457) has shown that in the rat after peritoneal dialysis, the plasma renin activity is highly elevated as well as the hematocrit values. Moreover, despite

plasma electrolyte changes, if the plasma volume is restored by glucose infusion or albumin, the plasma renin activity returns to normal values depending on the amount of the volume restored. In such cases no appreciable changes were noted in JG cell granularity (200).

Furthermore, Meyer et al (458) have reported in man, that an acute ethacrinic acid injection into normal subjects produced an increase in natriuresis, hematocrit, plasma renin activity and urinary volume. Moreover, if polyvinylpyrrolidone is perfused at the same time as the injection of ethacrinic acid, the plasma renin activity and the hematocrit do not increase, despite the natriuresis present. Thus these authors suggest that the renin secretion in such experimental conditions depends on the plasma volume.

2 - TUBULAR FLUID COMPOSITION AT THE MACULA DENSA LEVEL.

Various groups of investigators impressed by the already described, light microscopic characteristics or electron microscopic findings or histochemical characteristics of the juxtaglomerular complex, have suggested that the morphological activity of JG cells and renin secretion might be controlled in some manner by the intratubular fluid via the macula densa (10, 14, 30, 57, 140, 322). Stamey (459) reconsidering the morphological and physiological parameters, has emphasized that the macula densa could act as a highly sensitive part of the nephron which locally transmits and even magnifies changes in the concentration of sodium from within the tubule to the JG cells. Furthermore, he suggested that the location of the macula densa is probably at a point in the nephron where, at least during a water diuresis, the tubular urine flows the slowest, which would allow maximal time for the movement of sodium across the macula densa

to the JG cells. On the basis of this osmoreceptor theory of the macula densa, Stamey proposed that such a mechanism may explain much of the experimental data on sodium, juxtaglomerular granularity, and renal perfusion pressures. Moreover, renal hypertensive patients with a more marked renal ischaemia, tubular atrophy, and non filtering kidney on intravenous pyelography, failed to show any hyperplasia of the JG cells, concluding that it seemed likely that hyperplasia of the juxtaglomerular cells required the formation of glomerular filtrate.

However, patients who had been cured of their renal hypertension but who had a normal appearing renal cortex demonstrated hyperplasia of the JG cells as the only histological abnormality . Guyton et al (460) after deriving a series of equations and calculations from experimental studies, concluded that the autoregulation of blood flow into the kidney was most probably controlled by an osmotic feed-back at the site of the macula densa versus JG cells.

There are several lines of evidence which tend to implicate the macula densa in renin release in acute experiments. Vander and Miller (226) studied the effect of infusing osmotic diuretics, acetazolamide and chlorothiazide, intravenously into dogs. They found that these diuretics did not influence the base line level of renin secretion. However, when they infused their diuretics and then constricted the aorta above the renal arteries, they did not get the expected rise in renin output. Moreover, in other experiments, they constricted the aorta above the renal vessels and obtained an elevation in renin secretion . They then infused diuretics intravenously and found that the renin output dropped sharply. Since the changes in urinary sodium and

osmolarity were accompanied by slight or no variation in the renal blood flow and glomerular filtration, the authors concluded that renin secretion is not controlled by blood pressure per se, or intrarenal pressure, but by the flow or composition of intratubular fluid, probably at the level of the macula densa. White (461) has reported similar experiments in which either mercaptomerin or the renal intra-arterial infusion of 1 M NaCl or sucrose suppressed the renin release induced by reduction of renal arterial pressure. Recently Tobian (462) has emphasized the macula densa theory. He found in rabbits that when renin secretion is being stimulated by a narrowing of the renal artery an infusion of plasma with a high sodium concentration tends to diminish the secretion of renin compared to an infusion with a normal sodium concentration. In contrast, other investigators have observed that the administration of mercurials (463), fursemide (464) and ethacrynic acid (458) will increase renin release within 1-2 hours when the diuresis itself, is still present and macula densa sodium load is very likely greater than normal.

Moreover, chlorothiazide (465, 246) and fursemide (246) also cause increased JG granularity. Thurau's group (378-381) has provided another piece of evidence implicating the macula densa, even though there were no actual measurements of renin output in their studies, Through a micropipette, they infused solutions of sodium salts retrograde into the distal tubule. This exposed the macula densa to the infused solution, when the concentration of the infused sodium salt was around 90 mEq/liter, they noted a quick collapse of the proximal convoluted tubule, indicating that there had been

constriction of a preglomerular arteriole. If they infused solutions of sodium sulfate, or cholinechloride, or mannitol, or potassium salts it did not cause collapse of the proximal tubule indicating that osmolarity is not the critical factor. As the concentration of the infused sodium salt was progressively reduced below 90 mEq/liter, the effect was progressively reduced. If the sodium solution was injected into kidneys which contained little renin, only 1 out of 36 kidneys showed a collapse of the proximal tubule. They postulated that in the rat kidney a sodium-specific feed-back mechanism occurs between the tubular and vascular structures at the level of the JGA, which acts inversely on the sodium concentration in the tubular fluid at the macula densa cells and glomerular filtrate rate. They also suggested that the mechanism by which glomerular filtration is reduced might well be the local formation of angiotensin in the wall of the afferent arteriole which reduces filtration pressure by preglomerular vasoconstriction. On the other hand, Leyssac (466) and Morel (467) have not been able to repeat the experiments of Thurau's group. Tribe and Heptinstall (214) have reported evidence eliminating the role of the macula densa in JG cell activity. In rats with glomeruli whose tubular continuity has been interrupted by chronic pyelonephritis or renal trauma, JG cell granularity highly increased following adrenalectomy plus sodium supplement. Furthermore, in ischaemic transplanted kidneys, in which macula densa was not encountered, Endes et al (221) have reported the presence of a marked JG cells granularity. Demopoulos et al (143) reported that after destruction of the tubules by sodium tartrate visualizing only atrophic macula densa structures, the JG cell presented a well granulated pattern. Bohle and Walvig (40) found that some types offish (teleosts) have large amounts of JG granulated cells, despite the absence of distal

tubules (macula densa) in their kidneys. This observation has been recently confirmed by Perlow et al (161).

3 - HUMORAL SUBSTANCES

Several lines of investigation have suggested a "humoral" feed-back in relation to the JG cell (renin secretion). Masson (218, 220) and coworkers postulated that the "adrenal mineralocorticoids" might represent the feed-back substances regulating the renin-angiotensin system. They observed that the secretion of pressor material by the kidney, as estimated grossly by the rise of blood pressure following kidney grafts in nephrectomized rats, decreased rapidly after the administration of DOC and salt before the development of hypertension while the renin content of the kidney remained unchanged. Similarly, but more gradually, cortisone or cortisol administration decreased the liberation of the pressor material by the kidney.

Associated studies reported appear to be in favor of the inhibitory effect of adrenal steroids on the secretory activity of the JG cells. A near disappearance of JG cell granularity in the kidney is constantly reported in rats treated with DCA and salt (32, 138, 140) as well as a marked decrease in renal renin content (134, 209) and circulating pressor substance (473, 474). Adrenal regeneration markedly decreased the JGI (130, 469). However, in similar adrenal regeneration experiments, Dunihue failed to find any significant change in JG cell granularity (470). Since in these experiments at least three parameters are implicated, hypertension, salt and DCA, it is difficult to assess what is the specific factor. When DCA is administered alone no changes were noted in the JGI (32, 72, 130, 471) in blood renin (473, 474) or in the renin content of the kidney (134, 209).

Moreover, DCA failed to decrease the JGI in rats maintained on a low salt intake and even the JGI reported to increase as compared to control animals during this experimental procedure (32). However, others reported a slight decrease in the JGI (220) and in the renal renin content of rats during chronic treatment with DCA alone. It appears from the foregoing discussion that DCA in itself is practically devoid of any consistent inhibitory effects on the secretory activity of the JG cells. Fisher and Tamura (477) observed a decrease in the JGI in rats injected subcutaneously with aldosterone in oil for three weeks. A decrease in renal renin content was also reported in rats treated with aldosterone and salt (476). Fukuchi et al (475) noted only a slight decrease in the JGI in rats treated with aldosterone in water for two weeks while the animals remained normotensive. In patients with primary aldosteronism a decrease in the JGCC and granularity of the JG cells has been observed. (451, 452). ADecreases of angiotensin levels and plasma renin activity have also been reported in such patients (441-450). However, it is not possible to determine to what extent aldosterone alone is involved in this inhibition, since sodium balance and plasma volume are also disturbed.

The increase of JG cell granularity in adrenalectomized animals (211-214), the increase in the renin content of their kidneys and the increase of circulating pressor material (133, 198), strongly demonstrate a hyperactivity of the renin angiotensin system after bilateral adrenalectomy. Chemical inhibition of the steroidogenesis by metapirone (478, 479) or amphenone (480) is also accompanied by an increase in JGI. These conditions are associated with marked changes in sodium and blood volume and again it is difficult to explain such changes

in the renin angiotensin system by a simple absence of the adrenals.

Furthermore, in rats, hypophysectomy neither alters JG cells nor prevents their stimulation by sodium deficiency (53, 151). In contrast, ACTH was reported to increase the JG cell granularity in rat kidneys even in the absence of the adrenals (480). A parallel pattern has been observed in old rats between the decrease of hypothalamic neurosecretory material, JG cells, and atrophy of the adrenal zona glomerulosa (347). The somatotrophic hormone produces a decrease in JG cell granularity accompanied by hypertension and arteriolar necrosis (481, 482).

In reviewing the stimulus for the secretory activity of the JG cells, it has also been emphasized that the JG cells could act as "osmoreceptors", by changes in sodium blood plasma levels. Thus, Hartroft, (483) has reported that JG cells of rats continued to become hypergranulated in response to sodium deficiency despite injections of vasopressin to prevent a drop in blood volume. Similarly, in hypertensive rats with one clipped kidney, sodium deficiency did not lower blood pressure but granulation of JG cells rose in the "unclamped" kidney (485, 486). The same relation to the sodium level was found in DCA or cortisone-induced hypertension in which the elevation of blood pressure was equal but JG granulation and renin content were reduced in rats given high salt and increased in those on a low salt regimen (484). However, these experimental conditions are very likely associated with changes in tubular fluid and it is difficult to consider solely the sodium level at the site of the JG cells. Contraversial findings have been reported regarding potassium salts (151, 487).

Masson et al (488) have reported that hog renin injected for four days into rats, resulted in a marked decrease in renal secretion of pressor material while the renin content of the kidney remained normal. Kuhn et al (146) reported that extracts of dog kidney with high concentrations of renin injected to rats produce a decrease in JG cell granularity. The authors interpreted this finding as a suppressed activity of the JG cells. Furthermore, after two weeks the administration of angiotensin to the rat is associated with decreasing JG cell granularity (65, 390, 391, 489).

De Champlain et al (192) found that, in sodium depleted subjects with high levels of plasma renin activity, the infusion of angiotensin even at subpressor doses produced a highly significant decrease of the circulating renin. However, they did not find significant changes after infusion of aldosterone or pressor amines. The authors postulated that a feed-back occurs between the angiotensin and renin secretion. This finding was later confirmed by Vander in the dog (194).

4 - NERVOUS SYSTEM.

Although "denervated" kidneys are reported to secrete renin in response to various stimuli (406, 490), there are, however, several lines of evidence that strongly suggest the presence of a reflex control mechanism for renin release from the JG complex. As it has been previously described the JG complex has a rich nerve supply in various animal species. Elaut (491) found that carotid sinus denervation produced hyperplasia of the JG cells in dogs.

Taquini et al (202) reported that the renin content of denervated rat kidneys decreases markedly. Tobian et al (492) found also a decrease

in renal renin content and JG cell granularity of the unilateral denervated kidneys of rats.

Scornik and Paladini (493) found that infusions of norepinephrine in dogs increase the angiotensin arterial level slightly but significantly. Wathen et al (494) reported that infusion of norepinephrine into the renal artery produced an increased liberation of renin by the kidney. Acute degranulationof JG cells has been demonstrated under similar conditions with epinephrine infusions in rats (495).

Vander (496) demonstrated that direct electrical stimulation of the renal nerves increased renin release. Hodge et al (497) demonstrated increased plasma angiotensin concentration in dogs during non hypotensive hemorrhage and found that this release could be prevented by ganglionic blockade or local anesthesia.

Dunihue et al (498) reported that the increased JG cell granularity in rats on sodium dietary salt restriction was prevented by one injection of reserpine or by four injections of norepinephrine administered at the onset of salt restriction. Moreover subcutaneous injection of norepinephrine reduced low salt elevated JG cell granularity when given after 29 days of low salt intake.

PART II

INVESTIGATIVE SECTION

CHAPTER I

MATERIAL AND METHODS.

A) HUMAN SUBJECTS.

Surgical renal biopsies from patients with renal diseases were used.

B) ANIMALS.

a) Beavers. Seven adult beavers have been used. (Other data were not available).

b) Dogs. Three mongrel dogs, weighing 15-18 kg were used. They were fed on stock laboratory diet and tap water "ad libitum". Their kidneys were removed under light chloralose anesthesia.

c) Rats. Most of the experiments were performed in rats of both sexes, using various strains. 1) Sprague-Dawley strain from the Holzman farm and Robidoux farm. 2) Royal Victoria Hospital strain from Quebec farm. 3) Wistar strain from Brevannes farm (France). The experiments were performed only on animals which were in apparently good physical conditions without malformation, infection or spontaneous diseases. The animals were kept in laboratory cages from 4-10 days after their arrival, to give sufficient time for adaptation to the environment prior to experimentation. The average body weight of the animals, at the initia-

tion of the experiments, will be indicated in the description of each study. During the experimental periods, the rats were kept either in standard laboratory cages of five animals each, or in metabolic cages of one animal each. The standard diet, when not indicated otherwise, consisted of "Purina Fox Chow", and tap water, both "ad libitum".

C) DRUGS, DIETS AND SURGICAL OPERATIONS.

These will be indicated in the sections concerning the various studies in which they have been used or performed.

D) BLOOD PRESSURE MEASUREMENT.

In the adrenal insufficiency experiments, mean basal arterial blood pressure was measured indirectly without anesthesia, by tail plethysmography (E & M Apparatus). In other experiments, the blood pressure was measured under light pentobarbital anesthesia, by cannulation of the carotid artery and recorded on a polygraph (Grass Polygraph Model 5D).

E) MORPHOLOGICAL METHODS.

a) GENERAL DESCRIPTION.

All the morphological findings were evaluated on the basis of microscopic examination. Kidneys were removed under pentobarbital or light ether anesthesia, examined macroscopically and sectioned in a longitudinal plane through its hilus. The histologic specimens were preserved in a fixative chosen according to the staining technique to be used. Ten per cent neutral formalin, Bouin's solution and Helly's modified fluid (2/3 Zenker, 1/3 10% neutral formalin) were the fixatives usually employed.

After fixation and dehydration, the specimens were embedded in paraffin, and sectionned serially, at least, in three different section

levels, at the surface, at 100 and 200 µ deeper. Staining techniques were selected according to either the type of kidney elements to be studied or the expected pathologic changes. Various basic staining methods were used : 1) Hematoxylin-phloxine, 2) Weigert, 3) Periodic acid Schiff Reagent (PAS), 4) Masson's light green trichrome stain. 5) Periodic silver methenamine, 6) Periodic silver according to Laidlaw's technique, 7) Periodic silver trichrome stain.

The use of each staining method will be indicated in the description of each section.

b) HISTOLOGICAL PROCEDURES FOR DEMONSTRATING THE GRANULES OF THE JUXTAGLOMERULAR COMPLEX. IMPROVEMENT OF THE BOWIE PROCEDURE.

Our histologic work was oriented in order to find out a specific stain for the granules of the juxtaglomerular cells, permitting a marked contrast, and a satisfactory study of the whole kidney.

1) MATERIAL AND METHODS.

Kidneys from rats, rabbits, dogs and human subjects were used, although most of the work has been done in adults rats weighing from 150 to 250 g. In order to correlate the degree of granularity of the juxtaglomerular cells using various staining techniques, the Hartroft and Hartroft (32, 71) juxtaglomerular granularity index (JGI) was used. The staining method described by Wilson (31) was also used but without success since the alcoholic counterstains removed most of the stain. Satisfactory results were obtained with the modification of the Bowie (21, 22) technique as described by Cowdry (23), and utilized by Pitcock and Hartroft (29) to demonstrate granules in the juxtaglomerular cells. Then, we tried to improve this last technique in

order to get a more satisfactory contrast of the juxtaglomerular complex and to make this procedure shorter. The Alcian Blue (P.A.S. (35) method was also compared.

TISSUE FIXATION.

Fixation was accomplished in two ways : a) kidney fixation "in vivo" by arterial perfusion or b) by embedding the kidney once removed in the fixing fluid.

GLUTARALDEHYDE "IN VIVO" FIXATION (FOR LIGHT AND ELECTRON MICROSCOPY).

1.7% cold isotonic potassium phosphate buffered glutaraldehyde (pH 7.2 - 7.4) was perfused against the flow of blood through the abdominal aorta during 5-10 minutes, at a pressure of 135 to 145 mm Hg. Kidneys were removed and cut in halves, one half was placed in Zenker-formol 10% for 18-24 hours and the other half used for electron microscopic studies.

EMBEDDING FIXATION.

The volume of fixing fluid employed was about 20 times that of the tissue to be fixed. Kidney blocks of tissue about 3 mm in thickness were fixed in the following fixatives.

A) Acetone, 24 hours at pH 7.6.

B) Formaldehyde-fixatives : 1) Concentrated 40% formaldehyde solution at pH 3.6. 2) Formol-saline 10% (40% formaldehyde solution, 100 ml ; sodium chloride 8,5 gm ; distilled water 900 ml), pH 7.1. 3) Neutral 10% formalin (40% formaldehyde 100 ml, water 900 ml, calcium carbonate to excess), pH 7.2. 4) Alcoholic formalin (3 parts of alcohol 80%, 1 part neutral formalin), pH 7.7.

C) Chromates : 1) Harada modification of Ciaccio fixation (5% potassium

bichromate 16 ml, formalin 4 ml, glacial acetic acid 3 drops, pH 4.2, 48 hours in ice box) then mordant in 3% potassium bichromate 7 days. 2) Muller-formol, (potassium bichromate 20 gm in 1000 ml distilled water. Before use add 1 part of formalin 40% per 9 parts of the bichromate solution) pH 5.1, fixation 24 hours.

The terms formaldehyde, formalin or formol are employed interchangeably.

D) Picric acid: Bouin's fluid (trichloroacetic acid 2%, 40% formalin 500 ml, hot water 4000 ml at 60-70° C, picric acid to saturation), pH 1.7. 2) Duboscq Brasil solution, (80% alcohol 1500 ml, 40% formalin 600 ml, acetic acid 500 ml, picric acid 10 gm) pH 1.9. With both fixatives, the fixation time was from 6 to 24 hours.

E) Mercuric salts: 1) Stieve's fluid (76 ml saturated aqueous mercuric chloride solution, 40% formalin 20 ml, and glacial acetic acid 4 ml) pH 2.6. 2) Stieve's fluid modification (glacial acetic acid was replaced by trichloroacetic acid 2% or acetic acid 36%) pH 2.6, 24 hours fixation. 3) Zenker's solution and variants: stock solution "Zenker without acetic acid" (mercuric chloride to saturation 7 gm, sodium sulfate 1 gm, potassium bichromate 2.5 gm, distilled water 100 ml); a) Zenker with acetic acid: add 5 ml of glacial acetic acid for 100 ml of the stock solution, pH 2.4, fixation: 24 hours. b) Helly's fluid: add 5 ml of formalin 40% for 100 ml of the stock solution just before using, pH 3.5, 24 to 48 hours fixation. c) Zenker-formol 10%: 2/3 of the stock Zenker solution and 1/3 of formol saline 10% before use, pH 3.8, 24 to 48 hours fixation. d) Tris-buffered Zenker-formol 10% to pH 6 and pH 7.1, 24-48 hours fixation. e) Zenker-formol 10% with acetic acid (add before using 5 ml of glacial acetic acid for 100 ml

of Zenker-formol 10% solution), pH 2.3, 24-48 hours fixation. f) Tris-buffered Zenker-formol 10% with acetic acid to pH 6 or 7.1, 24-48 hours fixation. g) Zenker-formol 10% with trichloroacetic acid (add before use 5 ml of 2% trichloroacetic acid per 100 ml of Zenker-formol 10% solution) pH 2.7, 18-24 hours fixation.

After the fixation period, the specimens were washed in running tap water for 20 to 24 hours. Tissues were mounted in paraffin blocks and serial sections from 2.5 to 5 μ were cut.

PREPARATION OF THE STAIN STOCK SOLUTION.

1) One gram of Biebrich Scarlet was dissolved in 250 ml of distilled water and filtered through a rapid filter paper into a beaker.

2) Dissolve 2 gm of ethyl or methyl violet in 500 ml distilled water and filter a small amount at a time into the beaker containing the Biebrich Scarlet, with frequent stirring.

The end point of neutralization was indicated when a small amount of the mixture placed on a filter paper did not show any color (other than the precipitate in itself), or better, when a clear filtrate was obtained.

3) The mixture should then be filtered and the precipitate dried.

4) The stock neutral solution was made by dissolving 0.1 gm of the dried precipitate in 10 ml of 95% alcohol (23). Up to 100 ml of the stock solution can be obtained from one batch using ethyl violet and up to 200 ml using methyl violet if the end point is carefully determined.

STAINING TECHNIQUES.

1) Place paraffin sections in 3 successive xylol dish for 3 minutes each.

2) When mercuric salts were used in the fixatives, the sections should be placed in alcoholic iodine (0.5% iodine in 70% alcohol), 3 minutes,

rinsed in 60% alcohol and passed through sodium thiosulfate 5%, 3 minutes.

3) Tap water 5 minutes.

4) Mordant in 2.5% or 3% potassium bichromate at 37-40°C for 7-8 hours.

5) Rinse in distilled water.

6) Immerse sections for 14-16 hours in 20% ethyl alcohol to which has been added 22-25 drops of the neutral stock solution (the solution should be stirred before use) per 100 ml.

7) Blot sections with tissue paper (Kleenex).

8) Dip wuickly 2 or 3 times in 2 successive solutions of acetone to remove excess stain.

9) Differentiate in concentrated clove oil until section appears reddish purple.

10) Immerse sections for 1 to 5 minutes, depending on the degree of differentiation already obtained, in 1/1 mixture of xylol and clove oil. (If one wishes to verify under microscope, the juxtaglomerular cell granules should be deep violet and the elastica lamina of the vessel should be violet).

11) Rinse successively in 4 batches of xylol.

12) Mount with 1/1 xylol balsam.

2) RESULTS.

In order to get satisfactory results any fixed tissue should be mordanted in potassium bichromate for several hours before staining. The results obtained using different fixatives and staining the tissues with the Bowie modified method for the juxtaglomerular granulated cells are summarized in table 3. Figures 3 to 6 illustrate differences in fixation and stain with various techniques used.

TABLE 3

Results of the Bowie modified staining procedure, for JG granulated cells, with various fixation techniques.

FIXATION		RESULTS †
Fixatives	Time	JGC*
Glutaraldehyde "in vivo" perfusion	5-10 minutes	++
Zenker-formol 10% †	18-24 hours	++
Acetone	24 hours	-
Formaldehyde fixatives		
Formaldehyde 40%	24 hours	+
Formol saline 10%	24 hours	+
Neutral 10% formaline	24 hours	+
Alcoholic formaline	24 hours	-
Chromates		
Harada	48 hours	+
Muller-formol	24 hours	-
Picric acid		
Bouin	6-24 hours	+
Duboscq Brazil	6-24 hours	-
Mercuric salts		
Stieve's fluid	24 hours	-
Stieve's fluid modified	24 hours	++
Zenker with acetic acid	24 hours	-
Helly's fluid	24-48 hours	+
Zenker-formol variants		
Zenker-formol 10%	24-48 hours	++
Tris Buffered Zenker-formol 10%	24 hours	-
Zenker-formol 10% with acetic acid 36%	24 hours	++
Tris Buffered Zenker-formol 10% with acetic acid	24 hours	-
Zenker-formol 10% with tri-chloroacetic acid 2%	18-24 hours	++

* - no coloration of the granules.
\+ satisfactory results.
++ best results.
† In rat kidneys.

In glutaraldehyde, fixed tissues gave better results in the methyl-violet mixture (8 to 12 hours in the staining solution are sufficient). Microscopically, the renal parenchyma was very well preserved and was stained pink-violet. The elastic tissue of the vessels was colored deep violet, the same as the granules of the juxtaglomerular cells.

Acetone fixed tissues were not satisfactory in our hands. Formalin fixed material can be used to show the granular epithelioid cells, but it is recommended to use the mixture with ethyl-violet. However, the tissue fixation in general was very poor. Alcoholic formalin gave negative results. The Harada modification of Ciaccio fixation gave good contrast of the granules, similar to those obtained with Helly's fluid, but the parenchyma architecture was not well preserved and metachromasia was often observed. The Bouin fluid gave satisfactory results. However, the granules stained in light violet, -and are not well contrasted.

Mercuric salt fixatives gave good results; however, when glacial acetic acid was added to a relatively high concentration, the granules disappeared and in some instances, only one or two juxtaglomerular granulated cells were encountered in the whole kidney.

The modified Stieve's fluid replacing the glacial acetic acid by trichloroacetic acid or 36% acetic acid, gave in our hands very good results not only for the study of the juxtaglomerular complex, but also, as total kidney staining procedure. The parenchyma was dark pink-violet, the nuclei and nucleoli were very well contrasted, the brush border of the proximal tubules gave a pale pink-blue color, the basal membranes and the elastic tissue were stained in violet, the granules of the juxtaglomerular cells were stained in deep dark-violet. It is recommended for this fixation to use the

mixture with methyl-violet (Fig. 7, 15, 16).

Helly's fluid gave satisfactory results. However, this fluid becomes turbid and darker in color after 8 to 12 hours, with deposition of dark brown precipitate, making necessary to renew the fixative. The buffered Zenker-formol solution gave negative results. Using Zenker-formol 10% this brown precipitate is avoided and the kidney tissue is well fixed after 24 hours. It is possible to utilize indistinctly the ethyl-violet or the methyl-violet mixture. Microscopically, the renal tissue is uniformly stained, the proximal tubules are reddish purple, and darker than the distal tubules, (light reddish) the brush border and the glomeruli are magenta. The infolding membranes mitochondria complex are dark red (Fig. 3, 5). This is sometimes very useful for the identification of the macula densa. The tubular hyaline droplets are stained deep-purple. The elastic tissue of the vessels is violet and the juxtaglomerular cell granules, when present, will be deep violet or blue-purple. The granules of mast cells at the renal hilus appear light blue. Using the staining procedure described, the induced metachromasia of the juxtaglomerular granulated cells is avoided. Another variant of the Zenker-formol fixative with the trichloroacetic acid 2% gave very good results. The general stained pattern was similar as with Zenker-formol 10%. However, the nuclei were much better contrasted (Fig. 12, 13, 14). It was of interest that some cells of the renal pelvic epithelium showed small granules in its cytoplasma (Fig. 8, 11), stained in the same manner as the juxtaglomerular granules. These granules gave also the PAS reaction and they did not present metachromasia.

As it is shown in the table 4, the results obtained in the JGI of granularity using the ethyl violet or methyl violet mixtures as compared to

PLATE I

FIGS 3 to 6. Illustrate JG cells in the afferent arterioles and macula densa of kidneys of normal rats, comparing various histological procedures. x 670.

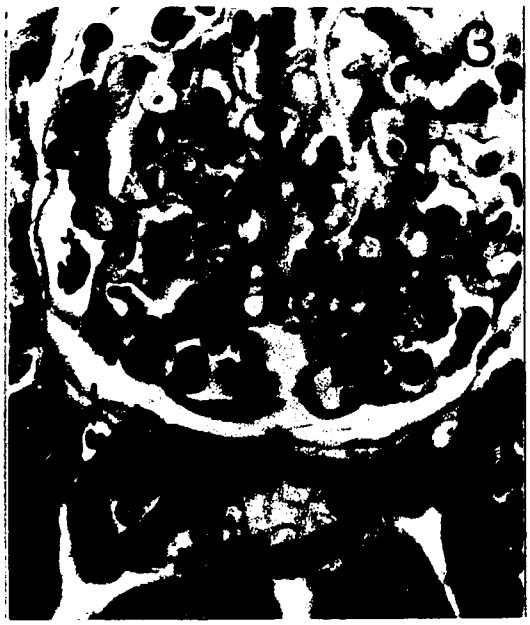
FIG. 3. Macula densa (md). Notice the absence of mitochondria infolding membrane complex. Beside the macula densa an afferent arteriole (Cut obliquely) showing JG cells. Blood capillary (Arrow). Zenker-formol 10% 24 hours. Bowie modified stain.

FIG. 4. Macula densa (md). JG cells (Arrow). Notice the shrinkage and the poor contrast of the parenchymal tissue. Helly's fluid 24 hours. Bowie stain.

FIG. 5. Macula densa (md), presenting the same characteristics as fig. 3. Notice a JG cell (Arrow) in the media of an afferent arteriole (cut obliquely). Proximal tub ules (PT). Distal tubule (DT). Zenker-formol 10% 24 hours. Bowie modified stain.

FIG. 6. Macula densa (md). Afferent arteriole (aa) showing the media JG cells (Arrow). Efferent arteriole (ea). Notice the difference in fixation of the tubules as compared to figures 3 and 5. Helly's fluid 48 hours. Bowie modified stain.

PLATE I



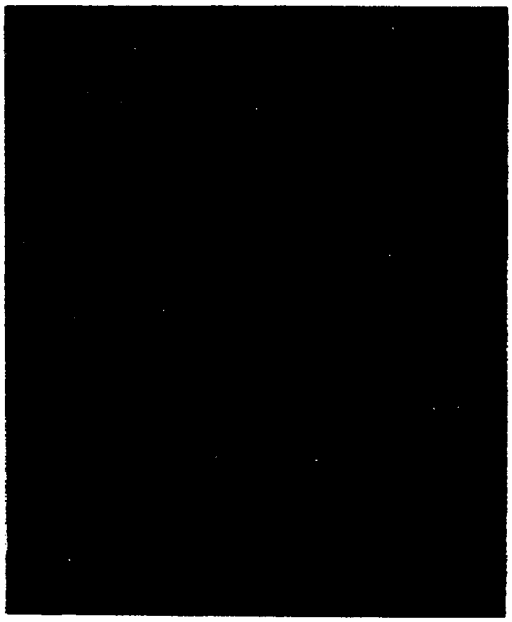
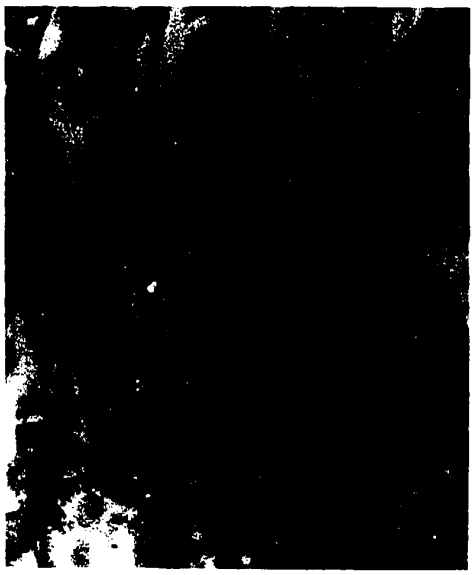


PLATE I



Alcian Blue PAS do not differ significantly ($P > 0.05$). However, in the Alcian Blue PAS Procedure, the pink granules are not so well contrasted when compared to the Bowie modified method.

In summary, the best results were obtained after a) fixation by glutaraldehyde perfusion and postfixation in Zenker-formol 10% and staining with the methyl violet mixture. b) Stieve's modified fluid using as stain the methyl violet mixture, or Zenker-formol 10% utilizing as stain the ethyl violet mixture. In any case the kidney sections should be mordanted in potassium bichromate for several hours before staining. These procedures gave also excellent results in rabbit, dog and human kidney.

Vacuole-like formations were occasionally found in JG granulated cells of normal rats (Fig. 10) as well as in patients with occlusion of the renal arteries (Fig. 7). Rarely the presence of granular cells was observed in the efferent arteriole on normal rats (Fig. 9).

TABLE 4

Comparison of the JGI* in the same rat kidney using three different staining procedures.

Number of rats	Staining procedures		
	PAS Alcian Blue JGI	Methyl violet mixture JGI	Ethyl violet mixture JGI
1	18	22	20
2	25	26	26
3	25	21	28
4	33	38	34
5	18	26	19
Mean ± SD	23.8 ± 7.88	26.6 ± 6.78	25.4 ± 6.16

* The evaluation of the JGI is described in page 96.

PLATE II

FIGS 7 to 11. The specimens were fixed in Zenker-formol 10%, 48 hours.

FIG 7. Kidney from a patient with severe obstruction of the left renal artery. Afferent arteriole (aa) showing in the media JG cells. Notice a vacuole-like formation in the media of the arteriole, with some granules (Arrow) making a prominence in the vacuolar lumen. Methyl violet mixture stain. x 40 x 15.

FIG. 8. Renal pelvic epithelium from a normal rat showing several granulated cells. At the left upper corner, the stroma, and at the right lower corner, the lumen. Bowie modified stain. x 400.

FIG. 9. Kidney from a normal rat showing JG cells in the media of the afferent arteriole (aa) and in the efferent arteriole (Small arrow). (Oblique cut). Macula densa (Large arrow). Bowie modified stain. x 670.

FIG. 10. Kidney from a normal rat. The arrow points to a vacuole-like formation in JG cells, similar to that of fig. 7. Bowie modified stain. x 1250.

FIG. 11. High magnification of the renal pelvic epithelium of the same animal as that of fig. 8. Arrow points to granules in the cytoplasm of a pelvic epithelial cell. Bowie modified stain. x 1250.

PLATE II

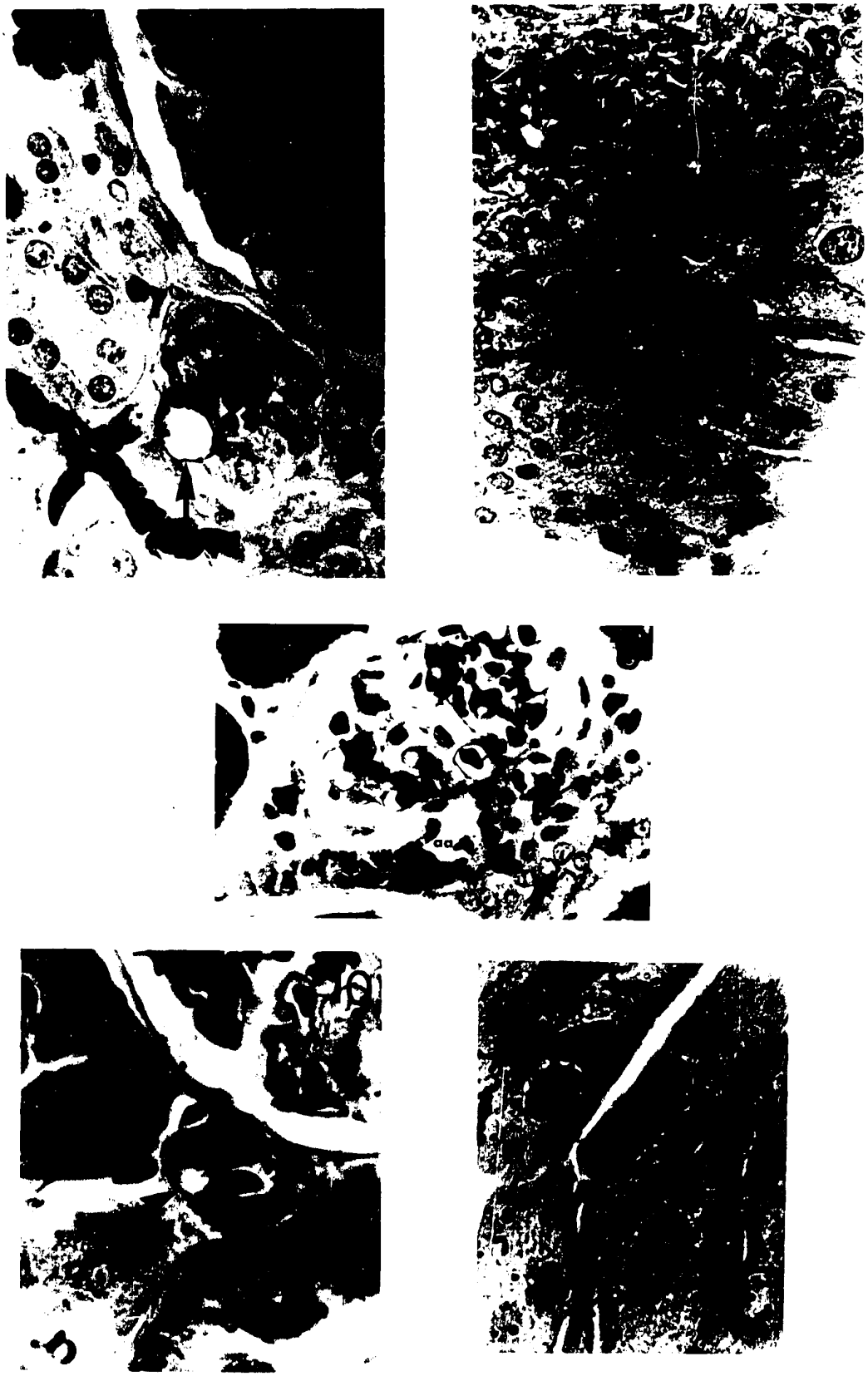
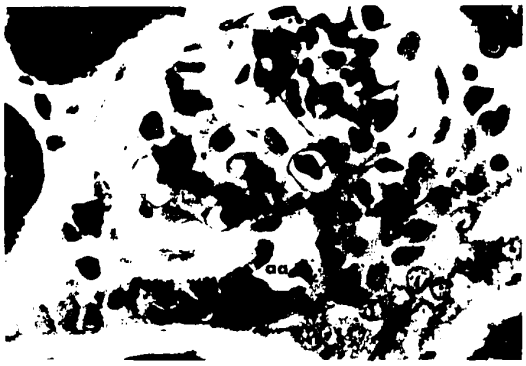


PLATE II



3) DISCUSSION.

The application of the glutaraldehyde perfusion fixation for light microscopic studies on the kidney and juxtaglomerular complex is very useful. This permits easy correlation with the electron microscopic studies in the same kidney, particularly in relation to the degree of granularity since the field for electron microscopy is too small for a semi-quantitative evaluation of the degree of granularity in large sections of the kidney.

The Harada fixative was highly satisfactory for the juxtaglomerular cell granules. However, it is a long procedure, taking 9 days and metachromasia of the granules is frequently observed. The acetic acid at relatively high concentration dissolves the granules, a fact already pointed out by Hennebert (28). However, at low concentration, it enhances the contrast as we have observed using the Harada fluid, the Stieve's modified fluid or the Zenker-formol 10% with trichloroacetic acid 2%. Using Zenker-formol 10% for 24 hours, no precipitate is formed and mordanting the tissue with potassium bichromate for 7 to 8 hours permits avoiding the JG cells metachromasia, which is seen when kidney tissue is fixed in Helly's fluid for 48 hours and mordant overnight in potassium bichromate as it has been described by Pitcock and Hartroft utilizing the Bowie technique. On the other hand, the procedure previously described, showed the following advantages : a) improvement and shortening of the fixation period, b) shortening of the mordant period, c) improvement of contrast by increasing the concentration of the staining . The use of methyl-violet gives the advantage that the amount of the precipitate obtained is two or three times greater as compared

to the one obtained with ethyl-violet. On the other hand, using glutaraldehyde or Stieve's modified fluid, the methyl-violet mixture gives better results than using ethyl-violet. Those procedures can be applied very satisfactorily on rabbit, beaver, dog and human kidney.

The absence of significant differences in the JGI of granularity using the neutral stains cited as compared to the Alcian Blue PAS procedure is in favor of the hypothesis of the glycoprotein nature of the granules. Furthermore, the cell granules observed in the epithelium of the renal pelvis seems to have also a glycoprotein nature. However, we do not know the meaning of these pelvic granulated cells, and further work is being carried out in order to elucidate their significance. They are different from mast cells and it is surprising that no previous description of such granular cells in the renal pelvic epithelium can be found in the literature.

Vacuoles in non-granulated juxtaglomerular cells have been seen most often in the human kidney (73). Goormaghtigh (82) observed the presence of vacuolated clear cells in JG cells of hypertensive rabbits and suggested that they represented part of a secretory glandular cycle. Hartroft (53) has seen similar vacuoles in dogs submitted to a sodium-free diet. However, there are no data concerning vacuolated granular epithelioid cells. The possibility exists that this change is due to alterations occuring during fixation.

However, in chapter VI of this thesis it will be shown in electron micrographs some structural changes that occur in juxtaglomerular granulated cells of the ischaemic kidney in rats that resemble those vacuole-

like formations already observed by light microscopy in non-granulated juxtaglomerular cells.

c) HISTOCHEMICAL METHODS.

We have studied the Triphosphopyridine Nucleotide Diaphorase (TPND); Glucose-6-phosphate dehydrogenase (G6PD); 6-Phosphogluconic dehydrogenase (6 PGD), and the α-Glycerophosphate dehydrogenase (GPD). The last three enzymes have been implicated in some metabolic processes occuring in the juxtaglomerular complex.

The methods for the visualization of the activity of these enzymes are based in the reduction of tetrazolium salts: 2,2'di(p-nitrophenyl) 5,5'-diphenyl-3,3'(3,3'-dimetoxy-4,4'biphenylene) ditetrazolium chloride (Nitro-BT), or 3-5 diphenyl-2-(4-5-dimethyl-thiazol-2-yl) Tetrazolium bromide (MIT) or 2,2', 5,5'-tetra-p-nitrophenyl-3,3'-(3,3'-dimetoxy-4,4'-biphenylene-tetrazolium chloride (Tetranitro-BT). These colorless and water soluble substances once reduced, become dark colored formazans which deposit at the site of enzymatic activity and in amounts depending on the activity.

1) GENERAL PROCEDURE.

Immediately after longitudinal section of the kidneys through their hilus, one half was frozen on metal holders with solid CO_2. When the whole kidney was available, the blocks were then transferred to a Cryostat (Slee, London, England.) at -20° C as soon as possible, usually within one hour, fresh frozen section 6 to 8 μ thick were cut at least in three different section levels of each kidney and mounted directly on cover-slips.

To ensure adherence to the cover-slips, the mounted sections were slightly warmed applying a finger on the cover-slips free surface for few

seconds, before being covered with 0.2 ml of incubating solution. The sections were incubated aerobically at 37° C for 30 minutes, with exception of TPND which was incubated for 15 minutes. After incubation in MTT-cobalt containing media, the sections were rapidly washed in 0.1 M HCL and water and fixed in 10% neutral formalin ; they were then mounted in glycerin jelly containing $CoCl_2$. The Nitro-BT and Tetranitro-BT treated sections of the formalin fixed tissues were washed in 10% ethanol for 10 minutes to dissolve most of the red reaction produced (arising from a contaminating monotetrazole) without causing appreciable shrinkage of the tissues. The sections after washing in water were mounted in glycerin jelly and those with cobalt chloride containing media were mounted in glycerin jelly containing $CoCl_2$.

2) REAGENTS.

The substances used for incubation media, their source and molecular weight (MW) are the following :

Reagent	MW
a) Substrates	
i) Reduced triphosphopyridine nucleotide (TPNH) Sigma Chemical Co. St-Louis.	--
ii) D-Glucose-6-phosphate disodium salt	358,2
D-Glucose-6-phosphate barium salt	521,7
D-Glucose-6-phosphate dipotassium salt Sigma Chemical Co., St-Louis.	390,4
iii) 6-phospho-gluconic acid sodium salt	342,1
6-phospho-gluconic acid barium salt	479,1
6-phospho-gluconic acid tri-monocyclohexyl-amonium salt	573,7

Sigma Chemical Co., St-Louis.

iv) DLα-glycero phosphate disodium salt 315,15

Sigma Chemical Co., St-Louis.

b) Buffers.

i) phosphate pH 7.3

Fisher Scientific Co., Montreal.

ii) Tris (hydroxymethylaminomethane) pH 7.4

7.6 - 8.0

Fisher Scientific Co., Montreal.

iii) Veronal pH 7.6

Merk and Company Ltd., Montreal

c) Co-enzymes.

i) Triphosphopyridine nucleotide (TPN) 734,4

Sigma Chemica Co., St-Louis.

ii) Menadione (2-methyl-1,4-naphtoquinone)

sodium bisulfate salt.

Sigma Chemical Co., St-Louis.

d) Tetrazolium salts.

i) MIT 415

Sigma Chemical Co., St-Louis.

ii) Nitro-BT 851

Sigma Chemical Co., St-Louis

iii) Tetranitro-BT ---

Sigma Chemical Co., St-Louis.

e) Activators.

i) Manganese chloride ($MnCl_2$) 197,92

Fisher Scientific Co., Montreal

ii) Magnesium chloride ($Mg\ Cl_2$) 203,33

Fisher Scientific Co., Montreal.

iii) Barium chloride ($Ba\ Cl_2$) 244,31

Merck and Company Ltd., Montreal.

iv) Cobaltous Chloride ($Co\ Cl_2\ 6H_2O$) 237,95

Fisher Scientific Co., Montreal.

f) Inhibitors.

i) Sodium azide ($Na\ N_3$) 65,02

Fisher Scientific Co., Montreal.

ii) Sodium cyanide (NaCN) 49,02

Fisher Scientific Co., Montreal.

iii) Sodium fluoride 41,99

British Drug Houses Ltd., Montreal.

3) INCUBATING MEDIA.

Various incubating media were prepared according to the original techniques described for each enzyme. However, modifications of these procedures based on the metal formazan techniques (292-295) will be described later on. The media were prepared freshly before use and had the following standard composition.

Triphosphopyridine nucleotide diaphorase (TPND)

Using MTT (Scarpelli et al (295)).

TPND Stock solutions.

MTT 1 mg/ml	2.5 ml
cobaltous chloride 0.5 m	0.3 ml
tris-buffer (0.2 M) pH 8.0	2.5 ml

distilled water	4.7 ml
polyvinylpyrrolidone	0.75 gm

Since the addition of cobalt results in marked lowering of pH. this solution must be adjusted to 7.2 with 0.2 M tris-buffer pH 10.4. This solution can be stored for 3 weeks at 0-4° C.

TPNH incubating medium.

stock solution	1 ml
TPNH	6 mg

Final concentration of reduced pyridine nucleotide is 1×10^{-2} M. These solutions are stable for 2 hours approximately.

The incubation period was 15 minutes at 37° C.

Using Nitro-BT (Farber (500)).

TPNH incubating medium.

Nitro-BT 1 mg/ml	0.7 ml
0.1 M phosphate buffer or tris 0.2 M pH 7.4	0.9 ml
TPNH 10 mg/ml	0.3 ml
Distilled water	3.0 ml

The incubation periods were 30 and 60 minutes.

4) HEXOSEMONOPHOSPHATE SHUNT DEHYDROGENASES.

Incubating medium (Hess et al 296, 293)

The medium had the following standard composition.

Substrate (1.0M)	0.1 ml
TPN (0.1 M)	0.1 ml

Respiratory inhibitor (sodium azide or sodium cyanide 0.1 M)	0.1 ml
Magnesium chloride (0.05 M)	0.1 ml
Tris-buffer pH 7.4 - 7.6	0.25 ml
Tetrazolium salt (1 mg/ml)	0.25 ml
Distilled water to make	1.0 ml
Polyvinylpyrrolidone (MW 10 000)	75 mg

For demonstrating the different dehydrogenases of the hexosamonophosphate shunt, the components of the above standard mixture was varied as follows :

A) Using MTT.

1) Glucose-6-phosphate dehydrogenase. Substrate, glucose-6-phosphate disodium salt, TPN, sodium azide, tris (hydroxymethyl) aminomethane buffer. $CoCl_2 - 6H_2O$ (0.5 M) 0.05 ml, sodium fluoride (0.01 M) 0.05 ml.

2) 6-phosphogluconate dehydrogenase. Substrate, 6-phosphogluconic acid barium or sodium salt (0.01 M) ; TPN ; sodium azide ; tris buffer 0.5 M 0.05 ml- $CoCl_2$.

B) Using Nitro-BT.

Glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase. The components of the medium were the same as using MTT except that $CoCl_2$ was not added in the media and for most purposes the respiratory inhibitor was sodium cyanide (freshly prepared and neutralized).

5) α-GLYCEROPHOSPHATE DEHYDROGENASE INCUBATING MEDIUM. (Hess and Pearse, 251, 293).

Substrate, sodium DL-glycerophosphate 1 M. Tris buffer 0.5 M, pH 7.4,

MTT 0.05 M, $CaCl_2$ 0.01 M, polyvinylpyrrolidone 75 mg/ml.

The individual constituants of the media, excluding the substrate, coenzyme, and respiratory inhibitor were buffered to pH 7.2 and stored at 0-4° C. The solutions were renewed every 4 weeks. The substrate, coenzyme and respiratory inhibitors were always freshly prepared and added to the stock solution just before use. The final pH of the medium was adjusted to pH 7.2.

6) IMPROVEMENT OF THE HEXOSAMONOPHOSPHATE SHUNT AND α-GLYCEROPHOSPHATE DEHYDROGENASES. HISTOCHEMICAL TECHNIQUES.

This section deals with further development and modification of the MTT-Co method (251, 295, 296) and is based on the metal formazan techniques (292, 293, 294, 295).

i) MATERIAL AND METHODS.

The present studies have been carried out with rat kidney ; the tissues were prepared as described in previous paragraphs. The time required for freezing of the tissue did not exceed 1 minute. After mounting on warm (room temperature) coverslips, the thawed sections were covered with 0.2 ml of the incubating medium and incubation was carried out for 30 minutes at 37° C.

In general, if not otherwise indicated, the pH of the buffer, the coenzyme concentration and the amount of the tetrazolium salts were kept constant, as indicated previously.

a) "Glucose-6-phosphate dehydrogenase" (G6PD)

The substrates used were : D-glucose-6-phosphate, barium, disodium and dipotassium salts. The medium considered optimal using Nitro-BT or Tetranitro-BT for demonstrating G6PD activity in rat kidney and mostly in the macula densa segment, is composed of the following reagents :

Substrate	
D-glucose-6-phosphate disodium salt : 0.2 M	0.1 ml
Respiratory Inhibitor	
Sodium azide : 0.1 M	0.1 ml
Coenzyme	
TPN 5 mg/ml	0.1 ml
Tetrazolium salts	
Nitro-BT 1 mg/ml or	
Tetranitro-BT 1 mg/ml	0.25 ml
Buffer	
Tris 0.2 M pH 7.6	0.25 ml
Activators	
Barium chloride : 0.67 M	0.05 ml
(Buffered at pH 7.2)	
Cobaltous chloride : 0.5 M	0.05 ml
(Buffered at pH 7.2)	
Glycolytic pathway inhibitor	
Sodium fluoride : 0.01 M	0.05 ml
Distilled water to make	1 ml
Osmotic protector	
Polyvinylpyrrolidone (MW 10.000)	75 mg

The final pH should be adjusted at pH 7.2.

b) "6-phosphogluconate dehydrogenase" (6PGD)

The substrates utilized were 6-phosphogluconic acid, barium, tri-monocyclohexylammonium and trisodium salts. The use of 6-phosphogluconic acid trisodium salt at a concentration of 0.2 M provided optimal conditions.

The reagent concentration of the incubating medium was exactly the same as in the glucose-6-phosphate dehydrogenase, except that sodium fluoride was not added.

The range of the substrate concentration employed, its final optimal concentration and the optimal final pH are summarized in table (5).

c) "α-glycerophosphate dehydrogenase" (GPD)

The optimal conditions using Nitro-BT or Tetranitro-BT were evaluated using various substrate concentrations from 0.01 to 0.1 M as final concentration. Of the buffers studied, veronal, phosphate and tris, the latter appeared best of the three.

Incubating medium.

D.L.α -glycerophosphate 1 M	0.1 ml
Buffer tris pH 7.6	0.25 ml
$CoCl_2$ 0.5 M	0.05 ml
Menadione 2 mg/ml	0.05 ml
Nitro-BT 1 mg/ mg or	
Tetranitro-BT 1 mg/ml	0.25 ml
Distilled water to make	1.0 ml
Polyvinylpyrrolidone (M.W. 10.000)	75 mg

Final pH should be adjusted to 7.2.

Sections incubated in a medium lacking substrate or coenzyme or boiled before incubation in ordinary medium served to verify the specificity of the various reactions.

ii) EXPERIMENTAL RESULTS.

The kidney sections were adequately stained for the three dehydrogenases activities studied, after 30 minutes of incubation period.

Considerable decrease of enzymatic activity together with loss of precise localization was observed after slow freezing of tissue blocks to -20°C. Repeated freezing resulted in a decreasing enzymatic activity.

a) "Glucose-6-phosphate and 6-phosphogluconic dehydrogenases".

The poor solubility of the barium salts employed as substrates was a limiting point, considering the range of substrate concentration that might be tested. The sodium salts of glucose-6-phosphate and 6-phosphogluconic acid gave us the best results, although, satisfactory results were also obtained using glucose-6-phosphate dipotassium salt for demonstrating the glucose-6-phosphate dehydrogenase.

To demonstrate the 6-phosphogluconic dehydrogenase, the 6-phosphogluconic acid tri-monocyclohexylammonium salt was also tested, without satisfactory results. The range of substrates concentration (using its sodium salts) tested, varied from 0.01 to 0.1M as final concentration, and for general practical purposes a final concentration of 0.02M appeared to be most adequate. The concentrations of the Nitro-BT and tetranitro-BT tetrazolium salts were kept always constant. In the case of G6PD, sodium fluoride was used as recommended by Hess et al (296) in order to inhibit the glycolytic pathway. When $BaCl_2$ or $CoCl_2$ were added to the media, the reactions were activated; however, the results were quite variable, mostly related to the location of the enzymatic activity at the macula densa level. However, the addition of combined $BaCl_2$ and $CoCl_2$, had a consistant activating effect in G6PD activity, giving a constant reaction in the whole kidney, and mainly in the macula densa. Other ions tested (Mg^{++}, Mn^{++}) at the same molar concentration had not such favorable influence on these dehydrogenases.

The optimal final $BaCl_2$ and $CaCl_2$ concentration are indicated in table (5). Reduction of the tetrazolium salts does not occur if either TPN or the substrate were omitted from the solution.

The histologic pattern of these dehydrogenases studied in the rat kidney as shown by the incubation medium just described, was similar to that reported by Hess et al. However, in contrast to the finding of these investigators, we found a slight activity of the hexosamonophosphate shunt enzymes in the proximal convoluted tubules, and a moderate activity in the straight portion of the proximal tubules. In addition, the epithelium of the renal pelvis showed a strong activity (Fig. 23).

The localization of these dehydrogenases in the cells showed a diffuse distribution and occasionally was in the form of dot-like deposits.(Fig. 19). No extracellular distribution was seen (Figs 17-21).

TABLE 5

Concentrations of substrates tested in media for demonstrating G6PD and 6PGD activity.

Substrate	Final concentration in medium		Activator final concentration in media	Buffer preferred	Final pH in media	
	Range tested	Optimal			Range tested	Optimal
Glucose-6-Phosphate disodium salt	0.01-0.1M	0.02M	$BaCl_2$ 0.0335M $CoCl_2$ 0.025M	Tris	6.0-8.0	7.2
6-phospho gluconic acid trisodium salt	0.01-0.1M	0.02M	$BaCl_2$ 0.0335M $CoCl_2$ 0.025M	Tris	6.0-8.0	7.2

The results obtained using $BaCl_2$, $CoCl_2$ and Nitro-BT as compared to those obtained with the MTT-Co showed the advantage of a more stable diformazan deposit, and the utilization of less amount of substrate.

b) "α-glycerophosphate dehydrogenases" (GPD)

The histological pattern of GPD in the rat kidney as revealed by the incubating media described, using nitro-BT or tetranitro-BT is parallel to that described by Hess and Pearse (251) using MTT. It is of interest that using MTT, nitro-BT or tetranitro-BT, the localization of the enzymes activity in form of dots at the vascular pole of the glomeruli was more frequently observed at the corticomedullary zones, in spite of the fact that this zone does not have the same number of granular epithelioid cells. In the outer cortex, the vascular pole exhibited very poor activity.

iii) DISCUSSION.

Nachles et al (292) using nitro-BT reported the activating effect of Ba^{++} on glucose-6-phosphate dehydrogenase. Furthermore, Hess et al (296) using MTT, had described the utilization of cobaltous chloride for demonstrating several dehydrogenases including the glucose-6-phosphate dehydrogenase. The activating effects of combined Ba^{++} and Co^{++} in the media containing nitro-BT or tetranitro-BT, for demonstrating the hexosemonophosphate shunt enzymes activity had not been reported previously, and was somewhat surprising.

These modified histochemical techniques have three main advantages:

1. The greater ease of reduction of nitro-BT or tetranitro-BT and its more stable diformazan deposit making studies possible after several weeks and even months, unlike the MTT formazan which crystallizes very rapidly at room temperature and with time the reaction decreases.

2. It is much more economical since the amounts of substrate and coenzyme used are lower than those required with the MTT-Co techniques.

3. It can be applied for the demonstration of the 6-phosphogluconic dehydrogenase, for which MTT-Co is useless since the final product is inhibitory of the reaction.

d) MORPHOLOGICAL EVALUATION.

In order to avoid subjective influences or impressions the morphological evaluations were done in many instances without knowledge of the experimental protocole. Furthermore, the reliability of the methods used were very often tested in blind basis. Thus, indices made in dozens of slides were verified two and three times, several days later and even after months. Minimal differences were observed (± 3) in both of the indices analyzed.

1) JUXTAGLOMERULAR GRANULATION INDEX (JGI).

The degree of granularity of juxtaglomerular cells "units" (JGI) was measured according to Hartroft and Hartroft (32) (An "unit" refers to a group of granular cells found together in the same arteriole, i.e. at the pole of the same glomerulus).

The entire cortical area of at least two sections from each kidney was covered systematically under the "high dry" magnification of the microscope. All glomeruli so encountered were counted, regardless of plane of section. Units of juxtaglomerular cells were recorded at the same time, and each unit was assessed for degree of granulation as follows :

Type I: Cells with only a few granules mostly clustered around the nuclei.

Type II: A few cells (1 to 3) with a greater number of granules than the preceding classification, which are scattered through the cytoplasm, but do

PLATE III

FIGS 12 to 14 illustrate JG cells in the afferent arterioles of rat kidneys. Zenker-formol 10% plus trichloroacetic acid 2%. Bowie modified stain. x 1250.

FIG. 12. Topographic view of the juxtaglomerular complex of a normal rat showing afferent arteriole (aa) with JG cells (Arrows). This unit of JG cells was classified as type I (see text). Efferent arteriole (ea). Lacis cells (lc) and the macula densa (md).

FIG. 13. Oblique section of the juxtaglomerular complex of a normal rat showing afferent arteriole with JG cells.(Small arrows). This unit was classified as Type II (text). Lacis cells (Large arrow) and the macula densa (md).

FIG. 14. Kidney of a rat adrenalectomized 2 weeks previously plus sodium supplement. Oblique section of an afferent arteriole showing JG cells (Arrows). This unit was classified as Type III. (See text).

PLATE III

not completely fill the cells.

Type III : A few cells (1 to 3) so densely packed with granules that the ground substances of the cytoplasm is obscured and the cells appear swollen.

Or a unit composed of many cells with degree of granulation corresponding to that described in Type II.

Type IV : More than three cells distended with densely packed granules.

Typical examples of each of the 4 types are illustrated in fig. 12 to 16.

The total recorded under type I-II-III and IV, were multiplied by the factors 1, 2, 4 and 8 respectively. The weighted totals were then expressed per 100 glomeruli to obtain the indices of granulation of juxtaglomerular cells.

The reliability of such an index has been discussed in detail by its authors (32), and although it was not considered to be a precise quantitative measure, it is a useful and practical method of estimating relative differences in granularity of juxtaglomerular cells units among groups of experimental animals.

2) GENERAL HISTOCHEMICAL EVALUATION.

The sections stained according to the histochemical techniques (TPND, 6PGD, GPD, G6PD) were examined with a Leitz light microscope. With MTT-Co and Nitro-BT-Co-Ba method, the granular formazan deposits are exclusively blue. With Tetranitro-BT, the granular deposit is brownish. With Nitro-BT-as-the hydrogen acceptor, two formazan are formed, a blue granular diformazan and a red monoformazan which gives a homogeneous red color not only to the cytoplasm but also to the nucleus. The red monoformazan was not considered in this study. In most of our experiments, the results will be expressed using

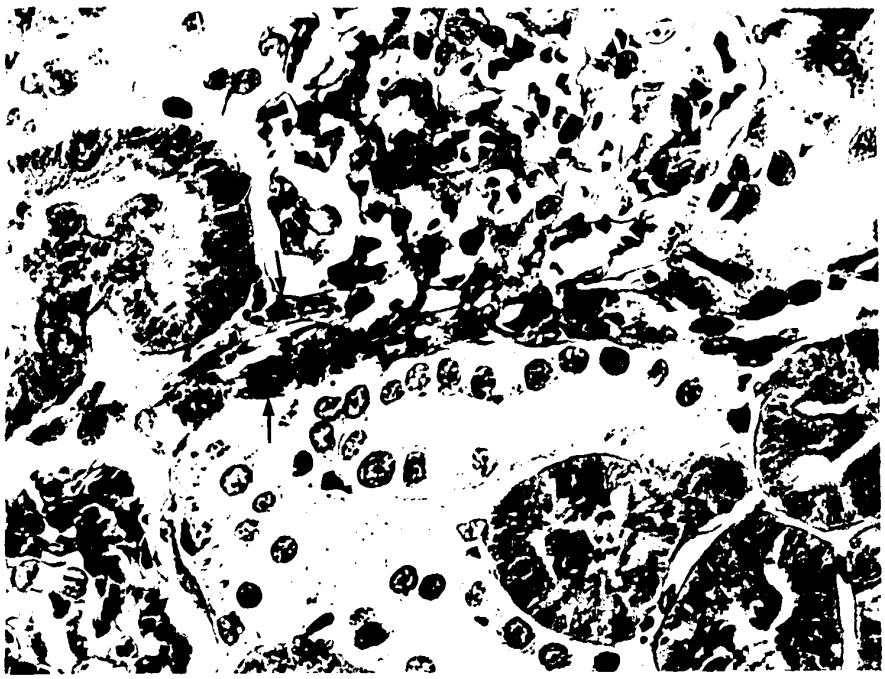
PLATE IV

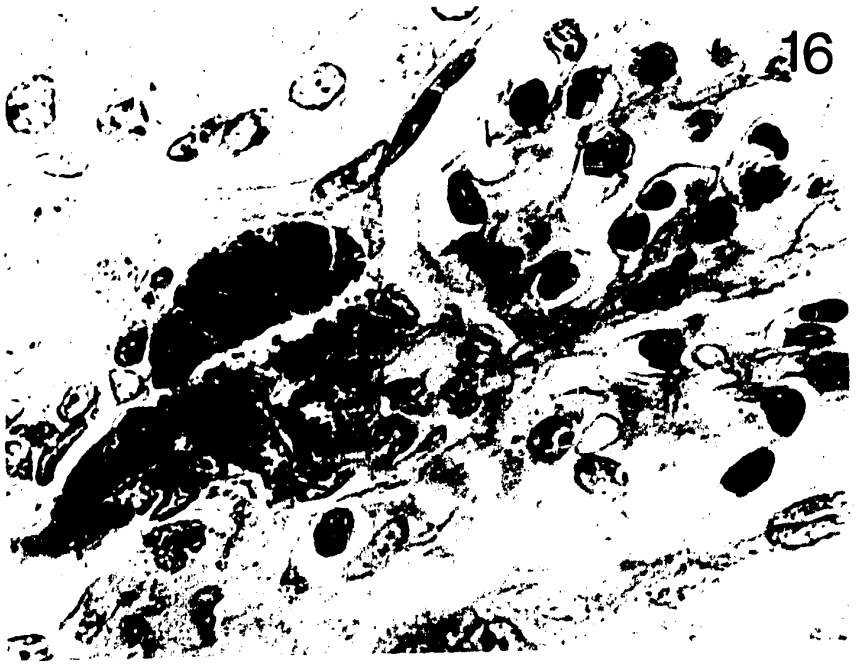
FIGS 15 and 16 illustrate JG cells after fixation in Stieve modified fluid (substitution of glacial acetic acid by acetic acid 36%, or trichloroacetic acid 2%), and stained by the methyl violet mixture.

FIG. 15. Kidney of a rat which received a low salt diet (Corn diet) for 1 month. Several JG cells (Arrow) are present in the afferent arteriole. This type of unit was classified as Type II. x 40.x 15.

FIG. 16. Kidney of a rat sacrificed 72 hours after mercuric bichloride injection. The JG cells seen here are distended and tightly packed with granules. Units of these characteristics were classified as type IV. x 80.x 15.

PLATE IV





the Nitro-BT-Co-Ba method. In the instances, where Nitro-BT or Tetranitro-BT were used, specific mention will be made.

The activity of the JGC, arterioles and macula densa segments in the sections were evaluated, according to the amount of formazan deposited, and were graded from 0 to 4, (0 indicating no detectable activity, and the numbers representing the degree of activity of the different enzymes : 1- weakly positive ; 2- moderatly positive ; 3- highly positive ; 4- very highly positive). Normal animals were used as controls in each experiment.

Although the intensity of the histochemical reaction does not lead in itself to precise quantitative measurement, we felt that the results obtained in each group of experiments correlated with the actual concentration of enzymes present, as all the sections were treated identically.

3) MACULA DENSA INDEX OF THE GLUCOSE-6-PHOSPHATE DEHYDROGENASE ACTIVITY (MDI).

The entire cortical area of each kidney section was analyzed systematically under the "high dry" magnification of the microscope. All glomeruli so encountered were counted, regardless of plane of section. Units of macula densa cells were recorded at the same time, and each unit was assessed for degree of granular formazan deposits as follows :

Type I : Cells with only a few formazan deposits mostly around the nuclei.

Type II : A few cells (1 to 4) with a greater formazan deposit, which are scattered through the cytoplasm mostly in the basal part but do not completely fill the cells.

Type III : A few cells (1 to 4) densely packed with formazan granules through all the cell in which the nucleus appears swollen.

Type IV : More than 4 cells of type III.

PLATE V

FIGS 17 to 20 illustrate various degrees of activity of the glucose-6-phosphate dehydrogenase in the macula densa of rat kidneys. Tetranitro-BT-Co-Ba technique. x 1250.

FIG. 17. Macula densa shows in some cells relatively few formazan deposits. This unit of macula densa cells was classified as type I. (see text). Glomerulus at the top right..

FIG. 18. Macula densa shows moderate formazan deposits. This unit was classified as type II (text). Glomerular arteriole (a) with its glomerulus at the right. Notice light zones at the basal region of the macula densa segment. That might well be related to the basal compartments shown in electronmicroscopy.

FIG. 19. High G6PD activity in the macula densa. This unit was considered as type III. Glomerulus at the left of the macula densa.

FIG. 20. The formazan deposits seen here are so numerous that cells appear distended. These units presenting such a strong activity are classified as type IV. Notice that in all described macula densa a granular component is present. However, the diffuse cytoplasmic reaction is predominant.

PLATE V

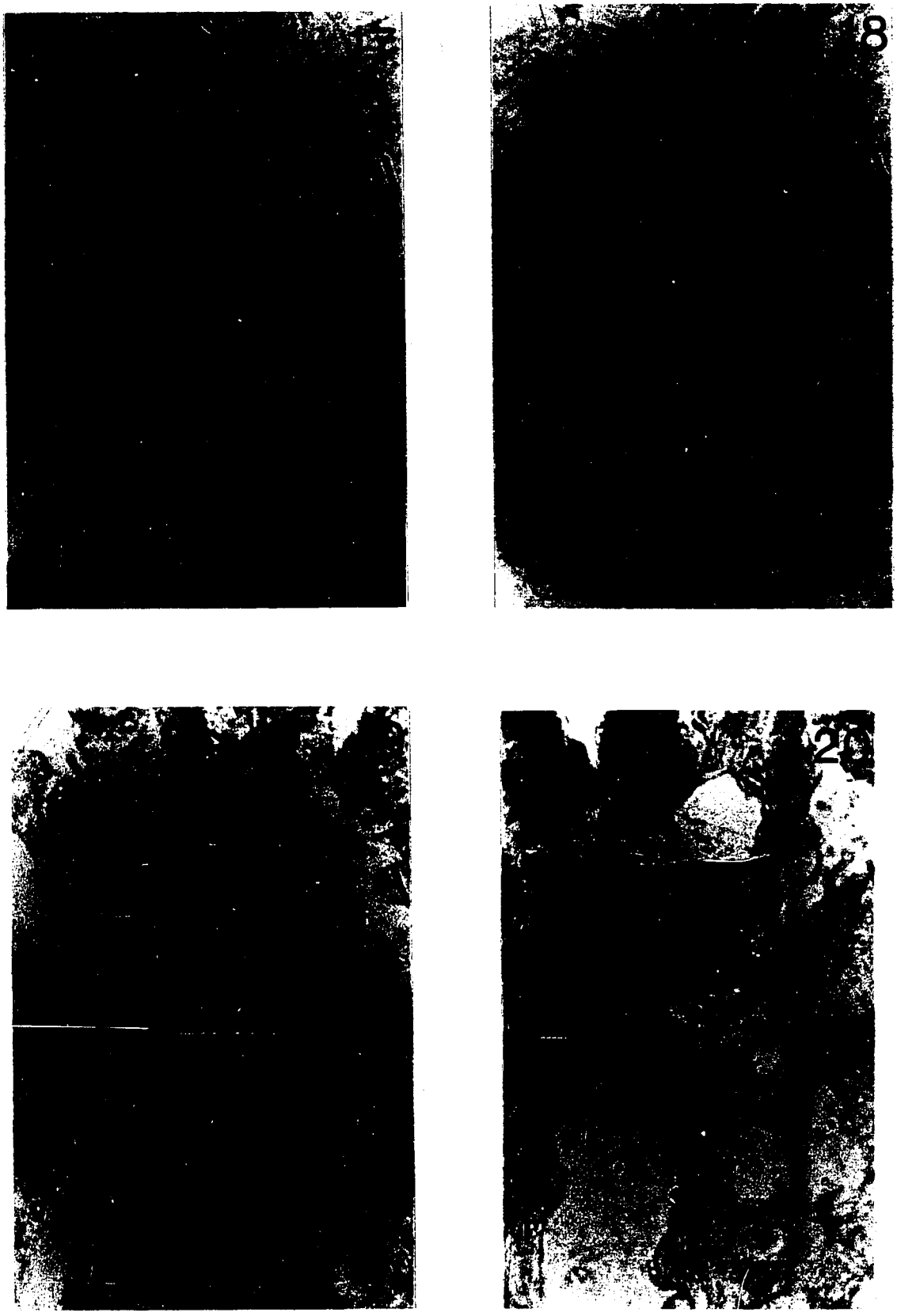


PLATE V

Such classification is somewhat artificial because of its crudeness and because transitional stages were encountered. But it is helpful in the same manner as the JGI of granularity of Hartroft and Hartroft as a practical guide in classifying large number of units. Typical examples of each of the 4 types are illustrated in figs. 17 to 20.

The total recorded under type I-II-III and IV were multiplied by the arbitrary factors of 1, 2, 4, 8 respectively, following the consideration of Hartroft and Hartroft for the granularity of the JGC. The weighted totals were then expressed per 100 glomeruli to obtain the indices of macula densa activity. Approximately 500 glomeruli were counted for each kidney.

In several instances, the identification of the morphological structures studied was performed under phase contrast.

Obviously the index described can be applied in the same extent to the 6-phosphogluconic dehydrogenase activity of the macula densa.

JGI and MDI values in normal animals are given in the next chapter.

e) ELECTRON MICROSCOPIC STUDIES.

The kidneys were fixed "in vivo" and removed under light ether anesthesia, in two ways : a) by local injection on the kidney surface of 2% cold buffered osmium tetroxide, and b) retrograde perfusion through the abdominal aorta, as has been described previously (p. 75) of 1.5% - 1.7% of isotonic potassium phosphate buffered glutaraldehyde, following the technique of Hatt et al (501). After fixation, small kidney blocks (1-2 mm) were cut. Glutaraldehyde perfused tissue was then fixed for 1 hour at 0-4° C in the same glutaraldehyde as that used for perfusion, rinsed 2 or 3 times in buffer, left 14-18 hours in buffer and then postfixed for 1 hour in 2%

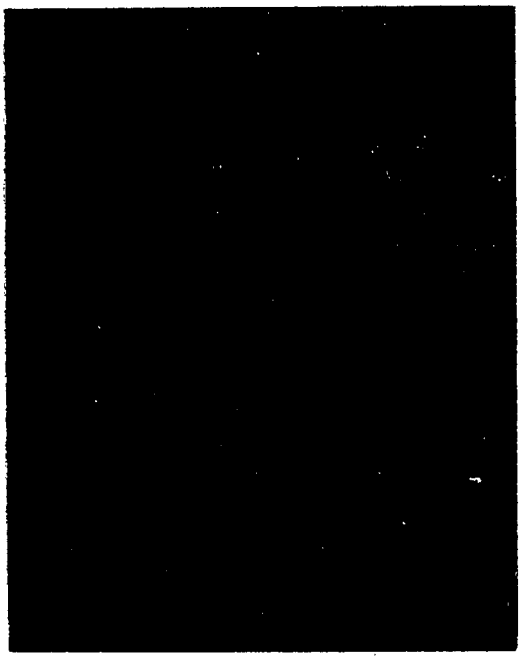
PLATE VI

FIG 21. 6-phosphogluconate dehydrogenase activity of a macula densa of a rat kidney (Type III). Glomerulus at the right of the macula densa. Nitro-BT-Co-Ba technique. x 500.

FIG. 22. Moderate α-glycerophosphate dehydrogenase activity in the JG cells (Arrow). Notice beside the JG cells, the macula densa. Glomerulus at the top right corner. Nitro-BT-Co Ba technique. x 500.

FIG. 23. Epithelial cells of the renal pelvis of a rat showing a high glucose-6-phosphate dehydrogenase activity. Nitro-BT-Co-Ba technique. x 670.

PLATE VI



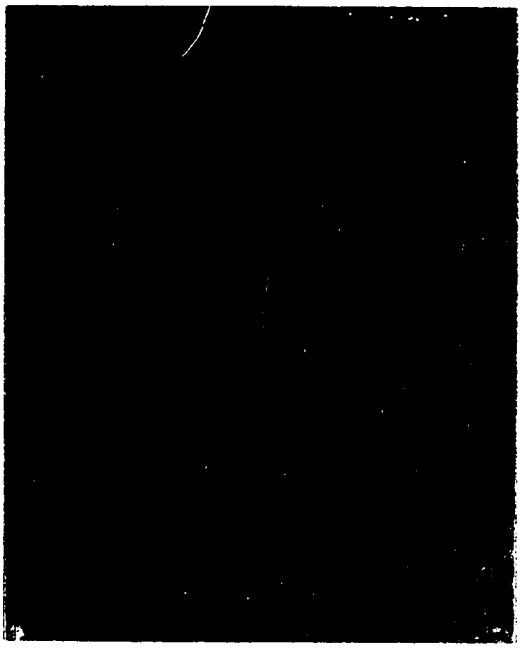


PLATE VI

buffered osmium tetroxide. Osmium injected kidneys were also postfixed for 1 hour in the same manner.

Fixed renal tissues were embedded in Epon 812 (502) after dehydration in graded alcohol concentrations. Thin serial sections (about 500 A°) were cut with glass and diamond knives on the Porter-Blum MT-1 microtome and mounted on copper grids; the sections were stained with lead citrate according to Reynolds' method (503) and examined and photographed in an EM-9 Zeiss electron microscope. About 1200 photomicrographs were taken from our experimental series.

Fort the visualization of fields with juxtaglomerular cells, thicker serial section (1 - 2μ) were cut with a glass knife for phase contrast microscopy and mounted with Canada Balsam on glass slides. Once the arterioles of the glomerulus were located, juxtaglomerular cells were identified by the presence of osmophilic granules.

F) METHOD FOR MEASUREMENT. PLASMA RENIN ACTIVITY (PRA)

Plasma renin activity (PRA) was measured in 0.1 ml of plasma from the jugular vein under light pentobarbital anesthesia, with a micromethod recently developed by Boucher et al (504). Results are expressed in nanograms (ng) of angiotensin formed during the 12 hours incubation period.

G) SIMPLE METHOD FOR PRODUCING ISCHEMIC ATROPHY IN KIDNEY OF RATS.

Four years ago, we made some experiments (unrelated to renovascular hypertension) in collaboration with Dr. M. Miranda in Selye's laboratory. We observed that, after complete ligature of the aorta between the two renal arteries in adult rats, about 60% of the animals developed an atrophic left kidney accompanied by a contralateral hypertrophic right kidney, frequent heart hyalinosis and in some instances, periarteritis nodosa-like

lesions mostly of the hepatic artery. This aorta ligature is frequently followed by hypertension. (see chapter VI).

a) MATERIAL AND METHODS.

Over 600 rats of both sexes of the Sprague-Dawley strain, weighing from 100 to 400 grs were used as indicated in table 6.

Surgical technique.

All operations were performed under ether anesthesia. The surgical interventions were carried out through incisions along the linea alba. In the first animal series, complete constriction of the aorta between the origins of the two renal arteries was accomplished using a silk-thread 0000. In some animals, two ligatures were made and the aorta cut in order to verify that no blood could pass through. In some animals, India ink was injected in the aorta above the coeliac trunk and superior mesenteric arteries in order to verify the collateral circulation. It should be noted that many rats have been lost when the left renal artery is arising from the aorta very near to the level of the right renal artery. In this case, meticulous attention must be taken where the ligature is placed in order to avoid occlusion of the left renal artery.

We have developed a modification in this technique in which the ureter of the ischaemic kidney is ligated and sectioned at the same operation time as the aorta ligature. This procedure was based in the endocrine kidney of Selye and Stone (505) in order to simplify their method making it reproducible in the hand of any research worker.

The animals presented in this chapter section were sacrificed at the end of the second week. Kidneys were removed, cut and fixed in formalin 10%, Zenker-formol 10% or Bouin. In addition to the general histological

procedures, sections were stained with Masson trichrome, PAS procedure, hematoxylin phloxin and Bowie modified.

b) RESULTS.

The results of these experiments are summarized in table 6. After the aortic ligature, all animals presented a paralysis of the posterior extremities that disappeared in a period of 1 to 2 hours.

The initial body weights of male and female rats are shown in table 6. The success of the procedures described appears to have a close correlation with the body weight (versus age) and to a lesser extent with the sex.

At the end of the experiments, the animals presenting an ischaemic atrophic kidney had a decrease in body weight of about 20% of the initial value. Mortality was approximately 20%.

Macroscopic findings.

Successful experiments were those of rats in which the aorta was completely constricted between the origins of the two renal arteries and with the left kidney diminished in size (Fig. 24,25). Color of kidney was pale pink instead of the normal reddish-brown. Endocrine kidneys with concomitant occlusion of ureter appear even smaller.

The right kidney was enlarged and pale yellow patches corresponding to areas of nephrosclerosis were often evident. Nodules of periarteritis nodosa-like lesions were often observed in the branches of the celiac artery, mostly hepatic. Hypertrophy and white infarctoid patches were frequently observed in the heart. Hypertrophy of the right and left adrenals was observed in all animals.

When India ink was injected in the aorta above the celiac trunk, no

TABLE 6

Experimental production of ischaemic atrophic kidneys in rats : percentage of success according to body weight and sex.

Number of animals	Body weight grs.	Sex	Experimental procedures			
			1. Complete aorta ligature between the renal arteries		2. Complete aorta ligature between the renal arteries and ureter ligature	
			Failure %	Success %	Failure %	Success %
50	100-120	M	95	5	100	0
		F	100	0	100	0
200	150-200	M	35	65	40	60
		F	45	55	45	55
50	220-240	M	27	73	33	67
		F	41	59	45	55
100	250-280	M	10	90	15	85
		F	28	72	25	75
200	320-400	M	8	92	85	15
		F	25	75	--	--

ink was observed to pass through the site of the ligature. This was checked by sectioning the aorta between two ligatures done in the territory between the origin of the two renal arteries. However, below the ligature, the aorta and left renal artery became filled with India ink, due to the development of the collateral circulation, mostly in the mesenteric territory (Fig. 24). The kidney sections revealed a corticomedullary hyperemia of the left kidney and small cyst-like formations were often observed in the right kidney.

Microscopic findings.

Histologic examination of the endocrine kidneys revealed a glomerular atrophy with enlargment of Bowman space and small glomerular tuft capillaries are filled with red blood cells. The tubules appear smaller. The proximal tubule epithelium loses its brush-border and the lumen of the tubules becomes merely collapsed and in some instances filled with proliferating well preserved epithelial cells. The nuclei of these epithelial cells were extraordinarily polymorphous in some regions, while in others they maintained a rather typical appearance. These changes were more marked in the corticomedullary region, in which mitotic figures were frequently observed. The cordon-like arrangement of epithelial cells observed in these non-urine-forming small kidneys, resembling the histological structure of the hypophysis and the parathyroid gland, led Selye and Stone to call them "endocrine kidneys". The morphological pattern of the small kidney produced in rats between 320-400 g. without ureter occlusion was the same as the endocrine kidney ; however, in several instances the tubular lumen was not completely occluded. No degenerative lesions or necrosis occur in the cortex of any of those kidneys. The right kidney showed extensive thickening, arteriolar necrosis and duplication of internal elastic lamina in small arteries

PLATE VII

FIG. 24. Macroscopic view of the abdominal cavity, showing the smaller left kidney as compared to the right one. Note the mesenteric vessels (Arrow), a few seconds after India ink injection (see text). Notice also the normal appearance of the intestines. 14 days after complete aortic ligature.

FIG. 25. Macroscopic view of the smaller left and large right kidney from the animal in fig. 24. Adrenals at the superior pole of the kidneys. The arrow shows the place of ligature.

PLATE VIII

FIG. 26. Area of an adenohypophysis of a normal rat. Masson's trichrome. x 300.

FIG. 27. Area from left "endocrine kidney". Note that the tubular lumina have disappeared and that the parenchyma take a similar appearance as the hypophysis (compare with fig. 26). Glomerulus at the top left. Masson's trichrome. x 300.

FIG. 28. Intralobular artery showing a mitotic figure (Arrow). Right untouched kidney. PAS. x 1250.

FIG. 29. Renal arteries and arterioles showing focal necrosis in the media and intima. Note the onion-like figure (Arrow). Right untouched kidney. PAS. x 300.

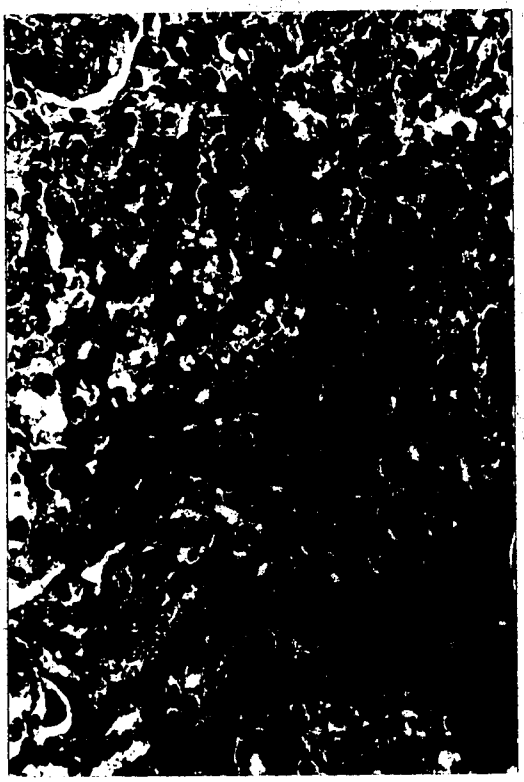
PLATE VII

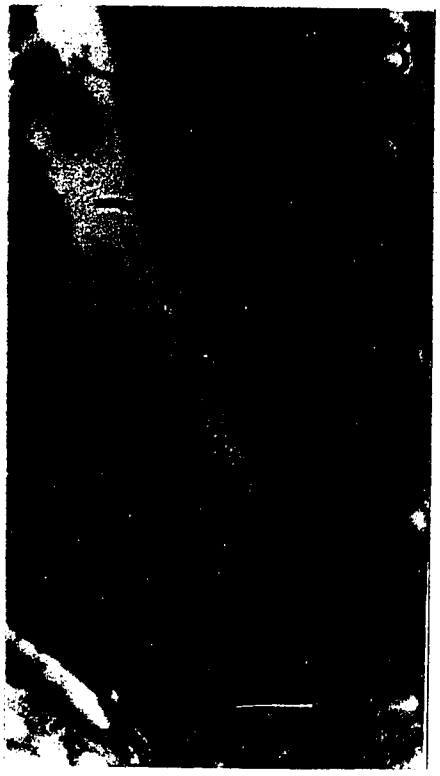
PLATE VII

24

25

PLATE VIII





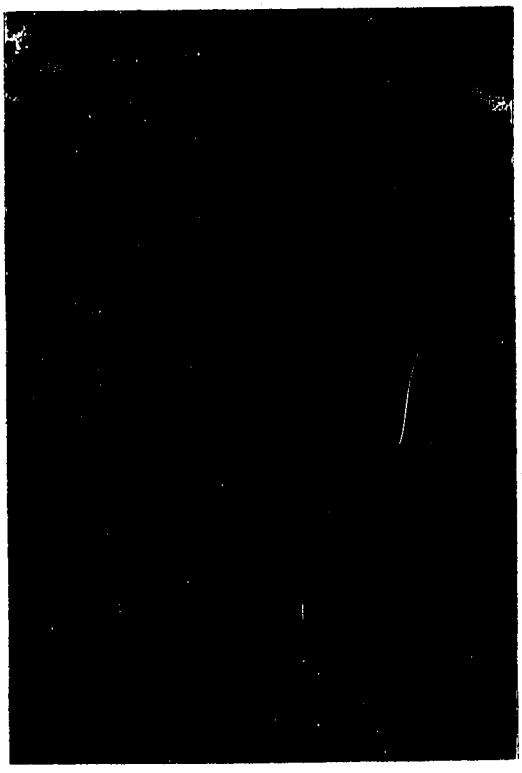
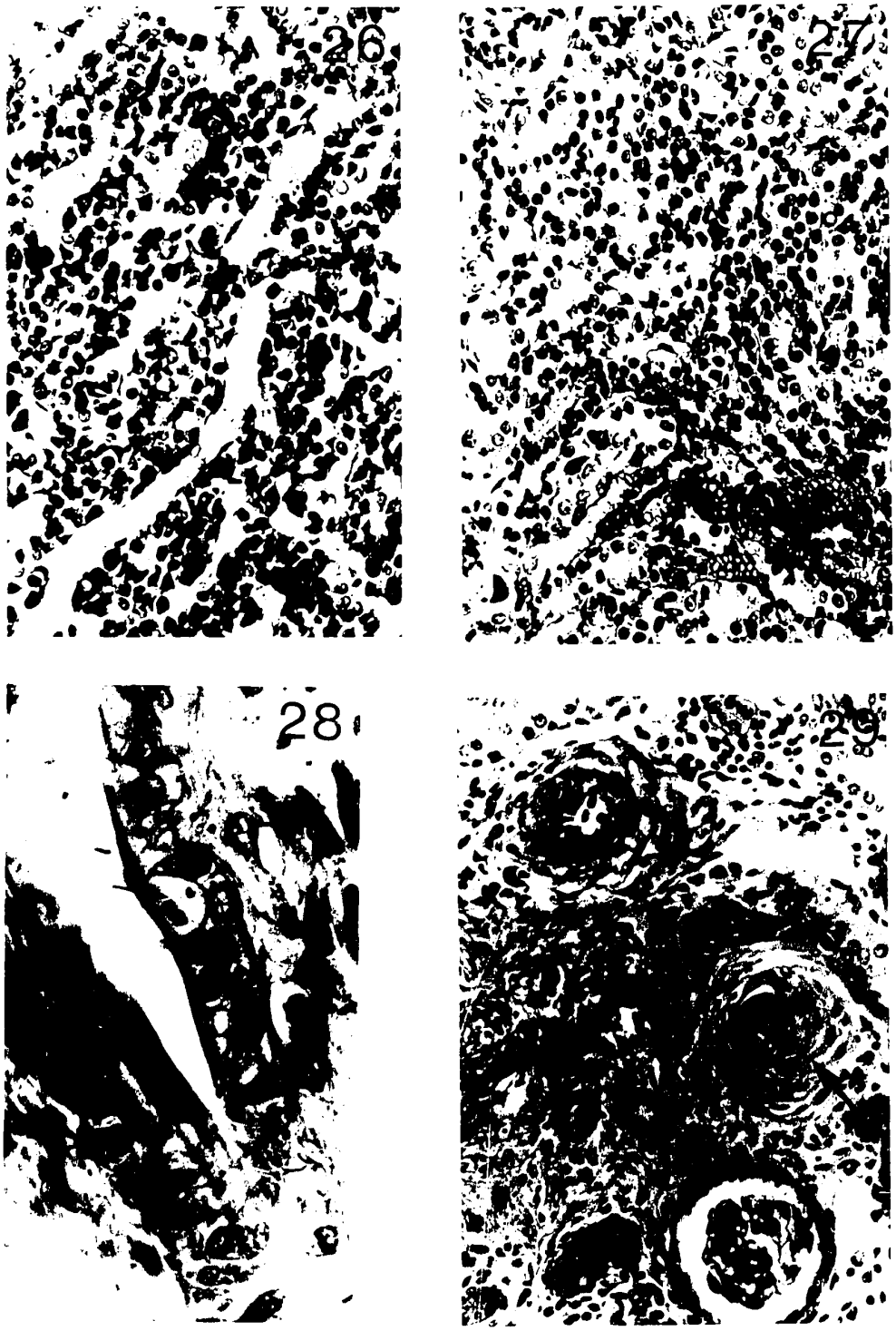


PLATE VIII



and arterioles (Fig. 29). Characteristics of malignant nephrosclerosis, such lesions were absent in the left kidney.

The blood pressure values obtained with these simple methods as well as the morphological changes in the juxtaglomerular complex will be described in the chapter VI (section A) of the thesis.

c) DISCUSSION.

Various methods are available for the production of ischaemic atrophic kidneys in the rat : the application of clips on the renal arteries (506) based on Goldblatt experiments ; the partial constriction of the aorta between the origin of the renal arteries with or without ureter occlusion (505) ; the ligation of the renal artery or branches of this artery (507, 508) ; cellophane perinephritis (509) ; rubber capsules (510) ; cellulose acetate cylinders (511) or a figure-of-eight ligature with an elastic band or silk (512). The various difficulties involved in these procedures are the relatively more complicated surgical techniques and the high percentage of the rats developing infarcted areas, necrosis, or infarction in the constricted kidney. The method of Selye and Stone (505) for the production of an "endocrine kidney" requires further comments. This method has the inconvenience of necessitating several stylets sizes (in order to constrict the lumen of the aorta approximately to the diameter of the stylet).

We found that such a procedure can be simply substituted by complete aorta ligature. It is interesting that the success of the method employed depends on the body weight (versus age) of the animal. Thus, a failure was obtained in young animals. Whether the adult rat kidney is more resistant to the ischaemia than that of the young rat, or they react in a different

manner in the development of the collateral circulation is a matter still to be clarified.

Ischaemic kidneys so obtained by simple complete aorta ligature are completely protected against nephrosclerosis even if the contralateral kidney of the same animal is severely affected.

As will be presented in chapter VI, using the simple methods described, a large number of rats developed hypertension making it valuable as an experimental model for the study of renovascular hypertension.

An extensive review of the literature describing the experience gained through the use of various techniques gives the firm impression that the pathological findings in the arterial system of the contralateral kidney have been essentially identical using any of these procedures. Masson, Hazard, Corcoran and Page (513), Toussaint, Wolter and Sibille (514), and Giese (515) also pointed to the similarities of lesions induced.

The major findings are always necrosis of the arteriolar wall, proliferative changes specially in the adventitia, thickening of the wall and fibrosis, pointing to a complex of destructive and reparative processes. The changes in the kidneys are of course dependent upon the nature of the renal intervention used. However, Halpert and Grollman (516, 517) described rats developing chronic hypertension after the application of a figure-of-eight ligature, without significant arterial changes.

As it is inferred from our experiments, one of the most striking facts was the marked development of the mesenteric collateral circulation in the rat. These observations bring out to us its possible role in renovascular hypertension. Thus, Blalock et al (518) demonstrated in dogs, that

unilateral renal artery constriction resulted in greater rise in blood pressure if the superior mesenteric artery was also previously constricted, obtaining in those animals sustained hypertension during one year. Schwartz (519) has reported in human beings that superior mesenteric artery insufficiency alone does not affect blood pressure but does predispose to hypertension in the presence of renal arterial abnormalities.

CHAPTER II

The purpose of this work is to demonstrate semiquantitatively the morphological variations and the distribution of components of the juxtaglomerular complex in various zones of the renal cortex in the rat, dog and beaver. The beaver was chosen as experimental subject since this animal possesses only short loops of Henle.

A) THE JG CELL GRANULARITY AND THE GLUCOSE-6-PHOSPHATE DEHYDROGENASE ACTIVITY OF THE MACULA DENSA IN NORMAL RATS : STUDIES ON SEX AND VARIOUS STRAINS.

a) MATERIAL AND METHODS.

1. Experiment 1. Eighty-five rats of three different strains (both sexes) weighing 150-200 gm were divided into two major groups (Tables 7 and 8).

a) Group I. Forty-eight male rats comprising this group were placed in three sub-groups : a) 30 rats of the Sprague-Dawley strain ; b) 12 rats of the Wistar strain ; c) 6 rats of the Royal Victoria Hospital strain.

b) Group II. Thirty-seven female rats were distributed in three sub-groups : a) 10 rats of the Sprague-Dawley strain ; b) 7 rats of the Wistar strain ; c) 20 rats of the Royal Victoria Hospital strain.

2. Experiment 2. Thirty-eight rats of two different strains (both sexes) weighing 150-200 gm were divided into two groups (Table 9).

a) Group I: 23 male were subdivided into two sub-groups : a) 18 rats of Sprague-Dawley strain, b) 5 rats of Royal Victoria Hospital strain.

b) Group II: 15 female rats were placed in two sub-groups : a) 5 rats of Sprague-Dawley strain ; b) 10 rats of the Royal Victoria Hospital strain

Kidneys were removed and each kidney was sectioned in half in a longitudinal plane through its hilus (see fig. 30). In experiment I, one of the two halves were fixed in Zenker-formol 10% for 24 hours. The kidney most symmetrically divised was selected and the ventral part embedded so that the plane surface was parallel to the microtome knife. Serial sections were cut at 2.5 to 5 μ, at least, in three different section levels with approximately 100 μ intervals. Thus, a total of 16 to 32 sections for each kidney were stained with the Bowie modified technique described previously.

In experiment 2, the kidneys were cut as in the experiment 1 and were studied following the histochemical procedures indicated previously for the demonstration of glucose-6-phosphate dehydrogenase activity.

The degree of granularity of juxtaglomerular cells was estimated using the JGI of granularity following a complete analysis of the kidney section from the lower to the upper perihilar zone. The degree of activity of the glucose-6-phosphate dehydrogenase was estimated using the MDI in the same way as for the JGI.

Over 42,000 and 15,000 glomeruli were counted respectively in experiments 1 and 2.

b) RESULTS.

Experiment 1. Results are indicated in tables 7 and 8. No statistically significant differences were encountered between males and females of the same strain ($P > 0.05$) or between males or females of the three different strains studied ($P > 0.05$). The range of JGI varied in the animal studied from 13 to 38 and the final mean JGI was 22 ± 5.86.

TABLE 7

The JGI in male rats of various strains.

EXPERIMENT I - Group I.

Number of rats	Strain		
	Sprague-Dawley	Wistar	Royal Victoria Hospital
	a) JGI	b) JGI	c) JGI
1	16	22	29
2	27	26	25
3	14	21	17
4	32	38	13
5	16	26	25
6	32	29	15
7	22	26	
8	18	18	
9	20	29	
10	26	31	
11	16	17	
12	23	28	
13	13		
14	16		
15	23		
16	20		
17	19		
18	25		
19	14		
20	23		
21	24		
22	32		
23	20		
24	21		
25	25		
26	19		
27	24		
28	25		
29	38		
30	20		
Mean ± SD	22 ± 6	26 ± 5.9	21 ± 6.5

Final mean ± SD 23 ± 6.17

TABLE 8

The JGI in female rats of various strains.

EXPERIMENT 1 - Group II.

Number of rats	Strain		
	Sprague-Dawley	Wistar	Royal Victoria Hospital
	a) JGI	b) JGI	c) JGI
1	18	23	17
2	23	20	15
3	37	24	20
4	17	28	22
5	31	17	13
6	35	19	14
7	22	25	20
8	17		16
9	20		19
10	21		26
11			21
12			24
13			22
14			17
15			17
16			19
17			21
18			25
19			32
20			25
Mean ± SD	24 ± 7.4	22 ± 3.8	20 ± 4.6
	Final mean ± SD 22 ± 5.5		

TABLE 9

Comparative studies of the G6PD activity of the macula densa (MDI) on sex and various strains

EXPERIMENT 2

Number of rats	Group I Male		Group II Female	
	Strain		Strain	
	Sprague-Dawley	Royal Victoria Hospital	Sprague-Dawley	Royal Victoria Hospital
	a) MDI	b) MDI	a) MDI	b) MDI
1	62	56	60	49
2	65	59	46	55
3	56	62	58	43
4	60	47	63	49
5	46	58	50	65
6	45			67
7	70			51
8	61			62
9	46			60
10	63			60
11	49			
12	67			
13	70			
14	51			
15	59			
16	52			
17	60			
18	67			
Mean ± SD	58 ± 8.34	56 ± 5.6	55 ± 7.1	56 ± 7.9

Final mean ± SD 57 ± 7.5

Experiment 2. The results are expressed in table 9. No significant differences in the MDI were observed between males and females of the same strain ($P > 0.05$) or between any male or female group of a given strain as compared to the other ($P > 0.05$). The range of the MDI varied in the animals studied from 43 to 70 and the final mean was 57 ± 7.5.

c) DISCUSSION.

Experiment 1. This study shows that no significant differences exist in the JGI in connection to sex or to the strain of rats investigated. However, regardless of the sex or the strain, a relatively large variation in JGI was noted from one rat to another. This variation might well be related to the various stressful situations of the laboratory, in different experimental periods. However, groups of animals under basal conditions and killed at the same time also show low and high JGI irrespectively of sex and strain. Rapp (356) reported that by selective inbreeding of mice with high and low JGI he found distinct lines with high and low JGI. Our experiment in rats shows that 13% of the animals had JGI from 30 to 38 (high JGI) and 34% from 13 to 19. Therefore, these findings strongly suggest that in the same manner as in the mice, different lines of rats may present low and high JGI.

Experiment 2. The purpose of this experiment was to test the reliability of the method developed for assessing the activity of the glucose-6-phosphate dehydrogenase of the macula densa (MDI) in the rat renal cortex. The method was not intended to be a truly quantitative one, but to be used in a comparative way in revealing relative differences in the amount of diformazan deposits in the macula densa among groups of experimental animals. This index possesses inherent limitations, due partly to the some-

what subjective evaluation by the investigator. Efforts were made to minimize this variable, by repeated analyses of several sections of the renal cortex for each animal without knowledge of previous exhibition nor of the type of experiment.

The absence of differences between sexes and strains might well indicate that the pentose phosphate shunt enzymes of the macula densa are specific to the rat species and that, at this anatomical level, the TPNH-regenerating system is more developed than in the rest of the nephron.

B) THE DISTRIBUTION OF THE JUXTAGLOMERULAR GRANULES AND OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE ACTIVITY OF THE MACULA DENSA IN THE RENAL CORTEX OF THE RAT.

a) MATERIAL AND METHODS.

The kidneys studied were from 17 male Sprague-Dawley rats weighing 200-250 gm. All the animals were fed on a diet of Purina Fox Chow and tap water "ad libitum", and were kept in individual metabolic cages for several days before being killed. The kidneys were sectioned through its longitudinal axis as it has been indicated previously.

The animals were divided into two groups :

Group I : 10 rats in which the sectioned kidneys were fixed in Zenker-formol 10% and stained with the Bowie modified method. Since preliminary studies revealed a definite regional distribution of the JG granules was noted, a thin line was traced through the middle of the kidneys sections dividing it into two zones, lateral renal cortex (LRC) and medial renal cortex (MRC) (see fig. 30). The JGI was calculated for each zone. In addition, the JGI was calculated in the glomeruli of the outer cortex (OC), (Renal cortex about 500-600 μ deep from the surface) and

in deeper glomeruli, at the corticomedullary zone (CM), identified by the numerous straight portion of the proximal tubule and the thick Henle loops. The JGI of the total cortex (C) was also counted.

Group II. 7 rats in which the kidneys were used for histochemical studies. The glucose-6-phosphate dehydrogenase activity was studied using the MTT-Co, and Nitro-BT, Co-Ba procedures. As in group I, the same kidney section division was established, and the MDI was calculated in LRC, MRC, OC, CM and C.

In both groups, over 500 glomeruli for each kidney were counted.

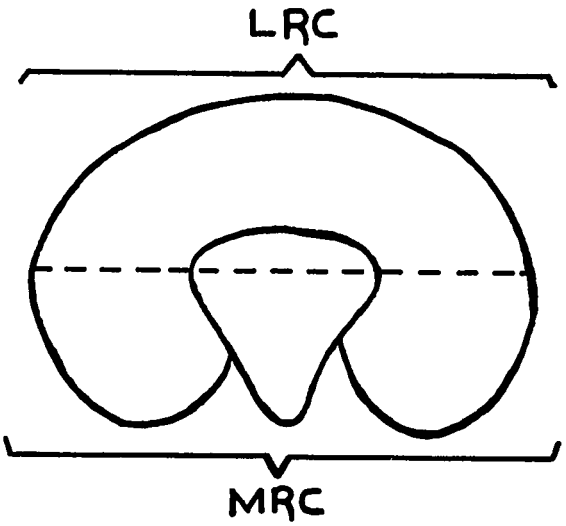


FIG. 30. Lateral renal cortex (LRC). Medial renal cortex (MRC).

b) RESULTS.

The results of the experiment are summarized in tables 10 and 11.

The total number of glomeruli usually contained in each section varied from 250 to 325, from which about 1/3 belonged to the corticomedullary region. The number of glomeruli of LRC as compared to MRC was approximately the same.

TABLE 10

The distribution of the juxtaglomerular granules in the renal cortex of the rat.

Number of rats	Lateral renal cortex (glomeruli) (LRC) JGI	Medial renal cortex (glomeruli) (MRC) JGI	Total outer cortex (glomeruli) (OC) JGI	Total cortico-medullary (glomeruli) (CM) JGI	Total cortex (glomeruli) (C) JGI
1	23	15	20	9	18
2	46	16	35	16	30
3	30	16	29	6	19
4	22	17	40	5	20
5	20	11	17	10	15
6	30	10	19	14	18
7	28	23	30	15	26
8	51	15	36	31	36
9	30	25	29	14	27
10	29	17	25	19	23
Mean ± SD	30.9 ± 10	16.5 ± 4.6	28 ± 7.7	13.9 ± 7.4	23.2 ± 6.5
	P < 0.001		P < 0.001		

TABLE 11

The distribution of the G6PD activity of the macula densa (MDI) in the renal cortex of the rat.

Number of rats	Lateral renal cortex (glomeruli) (LRC) MDI	Medial renal cortex (glomeruli) (MRC) MDI	Total outer cortex (glomeruli) (OC) MDI	Total cortico-medullary (glomeruli) (CM) MDI	Total cortex (glomeruli) (C) MDI
1	46	49	58	15	45
2	45	43	53	28	45
3	46	40	48	10	44
4	47	46	57	10	46
5	63	64	73	14	67
6	49	40	52	20	49
7	68	70	81	30	69
Mean ± SD	52 ± 9.4	50.2 ± 12.9	60.2 ± 12.1	18.1 ± 8.1	52.1 ± 10.9
	P > 0.7		P < 0.001		

Both juxtaglomerular index and macula densa index in the outer cortex as compared to corticomedullary region were significantly greater ($P < 0.001$). A significant difference was also observed when the JGI of the lateral renal cortex (LRC) was compared to the medial renal cortex (MRC). However, no differences were observed in the MDI when LRC and MRC were compared ($P > 0.7$).

c) DISCUSSION.

Juxtaglomerular index. The studies by Faarup (353), and Tribe and Heptinstall (214) are the two only previous observations on the distribution of the juxtaglomerular granularity in rats. The first author has reported in two selectively small zones of the renal cortex of the rats, (subcapsular cortex and juxtamedullary cortex from the middle and lower poles of the kidney), a higher JGI of granularity in the subcapsular than in the juxtamedullary cortex. Tribe and Heptinstall making tansverse kidney sections have described a higher JGI in the great curvature of the sections as compared to the perihilar cortex.

The purpose of this study was to analyze the total renal cortex in longitudinal kidney sections. In so doing, we have substantiated the findings of Faarup (353) by further confirming a significant difference in the JGI of glomeruli localized at different deepness from the cortex surface.

The JGI differences between the lateral renal cortex (LRC) and medial renal cortex might be well correlated with the observations of Tribe and Heptinstall (214) between the great curvature of the kidney cortex and the perihilar cortex.

Macula densa index. No previous work has been reported in the literature on the distribution of the glucose-6-phosphate dehydrogenase activity of the macula densa in any animal species.

The intimate anatomical association of the macula densa and the juxtaglomerular granulated cells have suggested to many workers a definite possibility of a functional relationship (322, 323, 226, 14). The present experiments reveal a significant difference of the MDI between the outer and corticomedullary cortex and fit well with the already described granular distribution and with Faarup's observation (353) of a high number of macula densa cells in the peripheral cortex as compared to the juxtamedullary cortex. However, the absence of correlation in the distribution of the JGI and MDI between the lateral and medial renal cortex must wait further investigation in order to evaluate its functional significance.

C) STUDIES ON THE JUXTAGLOMERULAR COMPLEX OF DOGS AND BEAVERS.

a) MATERIAL AND METHODS.

As in the rat, renal cortex of the dog was divided into lateral renal cortex (LRC) and medial renal cortex (MRC) (see Fig. 30). Then JGI was counted in both regions. Glucose-6-phosphate dehydrogenase using the Nitro-BT-Co-Ba method was also studied.

In both dog and beaver renal cortex the JGI was calculated in the glomeruli of the outer cortex (OC) (renal cortex about 1.5 mm deep from the kidney surface) and in deeper glomeruli (corticomedullary zone (CM)). In addition, the JGI of the total renal cortex (C) was also encountered in both dogs and beavers.

Over 3,000 glomeruli in the dogs and over 3,500 glomeruli in the beavers were counted.

b) RESULTS.

Dogs. Results are indicated in table 12 and figs. 31-33. Differences in the JGI of granularity between the outer cortex and corticomedullary cortex were significant ($P < 0.02$). JGI was also significantly higher in the lateral renal cortex (LRC) as compared to the medial renal cortex (MRC) ($P < 0.05$). The mean JGI of the total renal cortex was $11,6 \pm 2,1$.

The appearance of the juxtaglomerular complex observed in the kidneys of normal mongrel dogs is illustrated in figures 31 and 32. The granular epithelioid cells are found mostly in the media of the afferent arteriole and are larger than the adjacent smooth muscle cells. In some instances, they can be seen in the media of the efferent arteriole as it is shown in the figures 31 and 32. The "lacis cells" of Oberling and Hatt (14) present a round or oval nuclei and are smaller than the arteriolar granulated cells, Usually a clear "halo" around the nucleus is present in some of then.

The glucose-6-phosphate dehydrogenase activity of the macula densa is relatively poor compared to the activity of this enzyme in the proximal or other parts of the distal tubules. The diformazan deposits are in dot-like deposits and in diffuse forms. No activity was observed in the media of the afferent arteriole (Fig. 33).

Beavers. The results of the JGI in the outer cortex and in the cortico-medullary cortex are expressed in table 13. Differences between these two parts of the beaver renal cortex were significant ($P < 0.01$). The mean JGI of granularity of the total glomeruli counted in both regions was of $6 \pm 1,7$.

TABLE 12

The distribution of the juxtaglomerular granulated cells in the renal cortex of the dog.

Number of dogs	Lateral renal cortex (glomeruli) (LRC) JGI	Medial renal cortex (glomeruli) (MRC) JGI	Outer cortex (glomeruli) (OC) JGI	Corticomedullary (glomeruli) (CM) JGI	Total cortex (glomeruli) (C) JGI
1	19	7	11	2	10
2	18	10	20	5	14
3	14	7	13	3	11
Mean ± SD	14.6 ± 3	8 ± 1.7	14.6 ± 4.7	3.3 ± 1.5	11.6 ± 2.1
	$P < 0.05$		$P < 0.02$		

TABLE 13

The distribution of the juxtaglomerular granulated cells in the renal cortex of the beaver.

Number of beavers	Outer Cortex (glomeruli) (OC) JGI	Corticomedullary (glomeruli) (CM) JGI	Total cortex (glomeruli) (C) JGI
1	10	4	8
2	6	4	6
3	6	3	5
4	13	0	8
5	8	2	6
6	4	0	3
7	7	4	6
Mean ± SD	7.7 ± 3	2.4 ± 1.8	6.0 ± 1.7
	$P < 0.01$		

The morphological aspect of the beaver juxtaglomerular complex is very similar to the dog, as it is shown in the fig. 34. However, few granulated cells were encountered in spite of the epithelioid aspect of the cells of the afferent arterioles at the pole of the glomerulus.

C) DISCUSSION.

The purpose of this study was to compare the degree of JG cells granularity in two animal species in which there are marked differences in the length of the Henle loops. (521, 522, 597).

Our findings in dogs have shown that the JGI of granularity follows the same distribution pattern as in the rat. No previous works have been reported in the juxtaglomerular granular distribution in dogs, or beavers. As described earlier in the rat, a differential distribution of pattern of JG granularity was also observed in the dog when counting the granules in longitudinal sections. The counts in the lateral renal cortex (LRC) of the kidneys was significantly higher than those in the medial renal cortex (MRC). These findings are obviously important in counting granules in the rat and dog kidneys.

Since the results may not be comparable if corresponding regions of different kidneys are not analyzed, it might be asked whether this indicates a differential function in various areas of the renal cortex. As previously described in this thesis it has been shown that kidneys of mice (48), rats (353), rabbits (354) and humans (355) have a great number of juxtaglomerular granulated cells in the subcapsular or peripheral cortex as compared to the juxtamedullary or corticomedullary ones. A close correlation has also been demonstrated with the amounts of renin in these two cortical zones both in rabbits (354) and in humans (355). Friedberg (48) has postulated that

PLATE IX

FIGS 31 and 32. Juxtaglomerular complex from a normal dog. Several juxtaglomerular cells filled with granules (Arrows), in the afferent (aa) and the efferent arterioles (ea). Lacis cells (lc). Beside the efferent arteriole the macula densa can be noticed in both figures. Zenker-formol 10%, trichloroacetic acid. Bowie modified stain. x 500.

FIG. 33. Slight glucose-6-phosphate dehydrogenase activity in dog macula densa (Large arrow). Glomerulus top right. Afferent arteriole (Small arrow). Nitro-BT-Co-Ba technique. x 500.

FIG. 34. Kidney from a normal beaver. Juxtaglomerular cells filled with granules (Small arrow). Macula densa (Large arrow). Helly's fluid, 48 hours. Bowie modified stain. x 670.

PLATE IX

PLATE IX

such variation is related to the length of the loop of Henle. The superficial glomeruli have short loops and the length gradually increases the deeper the glomerulus is from the surface. Most of the animals.have both short and long loops of Henle (rat have 28% of long loops). Some animals have either long loops of Henle such as the dog and the desert rodent (521, 522) psammomys, or all short loops as the beaver. Our observations summarized in table 12-13 indicate that the greater number of juxtaglomerular granulated cells are not correlated with the length of the Henle loop. However, a zonal variation in the JGI is present and further studies are needed in order to interpret these results.

Bárajas (16) reported the presence of granulated cells in the efferent arteriole in adrenalectomized rats and there are no previous data concerning the presence of granular cells in the efferent arterioles of the juxtaglomerular complex in normal dogs. Our findings showing the presence of granular epithelioid cells in the media of the efferent arteriole in normal dogs and rats (see chapter I, fig. 9) support the concept of the continuity of the granular epithelioid cells with the mesangium (16, 339, 340, 341).

CHAPTER III

A) THE JG CELL GRANULARITY, MDI OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE ACTIVITY AND PLASMA RENIN ACTIVITY UNDER VARIOUS ELECTROLYTE INTAKES IN THE RAT.

a) MATERIAL AND METHODS.

Salt-deficient diets :

Three sodium free salt mixtures were used :

a) Hartroft and Hartroft (32) sodium chloride-deficient diet consisted of the following : casein 18%, fibrin 1%, zein 1%, celluflour 2%, salt mixture 4%, vitamin mixture 1%, sucrose 60,65%, beef fat 10%, corn oil 2%, choline chloride 0,35%, cod liver oil concentrate 0,015%, alpha tocopherol 0,01%. The salt mixture contained : $CaCO_3$, 11% ; $CaHPo_4.2H_2O$, 32,5% ; K_2HPO_4, 27,5% ; $MgSo_4$. 3 1/2 H_2O, 10% ; celluflour, 15% ; $FeC_6H_5O_7$, 3% ; trace elements 1%.

b) Nutritional Biochemicals Corporation (NBC) (Cleveland, Ohio) : sodium deficient test diet composed of sucrose 72%, "vitamin free" casein 18%, Butter fat (salt free) 5%, sodium free salt mixture 5% which contained calcium carbonate 37,5%, potassium phosphate monobasic 28,4%, potassium chloride 18,9%, ferric citrate 9,5%, magnesium oxide 3,8%, copper sulfate 1,9%. The diet was supplemented with vitamins.

c) The third type was simply corn, which contains only traces of sodium chloride (601).

Animals.:

90 albino male rats of the Sprague-Dawley and Wistar strains weighing 150-250 g. were used.

Experiment 1. "Sodium restriction": 47 rats were divided into two major groups (Tables 14, 15).

Group I. Duration of experiment 3 days: 14 rats comprising this group were placed into four subgroups.

Subgroup Ia: 5 rats. They were fed with the NBC sodium deficient diet and given distilled water as drinking fluid.

Subgroup Ib: 2 rats. They were fed with the NBC salt-deficient diet supplemented with sodium chloride 0.5% in 5% glucose in their drinking water. This group served as control to the subgroup Ic.

Subgroup Ic: 5 rats. They were fed as subgroup Ib but did not receive the supplement of sodium chloride in their drinking water.

Subgroup Id: 2 rats. They were deprived of food for 3 days, receiving as drinking fluid tap water.

Group II. Chronic sodium restriction. 28 rats were subdivided into 5 subgroups, in which all rats were of the Sprague-Dawley strain except the IIc of the Wistar Strain. All the animals received distilled water as drinking fluid.

Subgroup IIa: 6 rats, fed with the Hartroft and Hartroft diet during 1 month.

Subgroup IIb: 4 rats, fed with the NBC salt deficient diet during 1 month.

Subgroup IIc: 5 Wistar rats, fed with corn diet.

Subgroup IId: 7 rats, fed with NBC salt-deficient diet during 2 months.

Subgroup IIe: 6 rats, same as the subgroup IId, but for 3 months duration.

Experiment 2. "Electrolyte loading": 32 rats were fed with the standard laboratory purina. They were divided into three groups (Table 16).

Group I: 12 rats received 1% sodium chloride in their drinking water during 1 month.

Group II: 10 rats received desoxycorticosterone acetate DCA (Percorten, Ciba) 1 mg/kg, injected subcutaneously. They were then subdivided into two subgroups.

Subgroup IIa: 4 rats which were given the hormone for 7 days.

Subgroup IIb: 6 rats with the same treatment for 21 days.

Group III: 10 rats from two different strains formed this group. They received 2% potassium chloride in their drinking water during 1 month. The animals were subdivided into two subgroups in relation to their strain.

Subgroup IIIa: 5 rats of the Sprague-Dawley strain.

Subgroup IIIb: 5 rats of the Wistar strain.

In addition, 10 intact male Sprague-Dawley and 6 male Wistar rats weighing 150 to 250 g. served as control animals. They were fed with the standard laboratory diet and tap water "ad libitum".

Morphological procedures: Kidneys which were removed one minute after the blood sampling for PRA measurement were sectioned, fixed and stained as previously described. One half served for histological and juxtaglomerular granules staining, and in some animals, the other half was used for determination of the glucose-6-phosphate dehydrogenase activity of the macula densa using the Nitro-BT-Co-Ba method. Semiquantitative analyses of granularity and enzymatic activity were done using the JGI and MDI techniques.

TABLE 14

EXPERIMENT 1

GROUP I. Sodium restriction for 3 days.

Sub-group	No of rats	Procedure	Body weight	JGI	G6PD MDI	PRA
Ia	1	Fed NBC salt-deficient diet + distilled water	↓ - 8%	7		25
	2			9		17
	3			19		16
	4			25		9
	5			9		--
Mean ± SD				13.8 ± 7.8*		16.7 ± 6.5**
Ib	1	Fed NBC salt-deficient diet plus 0.5% supplement NaCl in 5% glucose	↑	20	50	15
	2			22	49	12
Mean ± SD				21 ± 1.4**	49.5 ± 0.7**	13.5 ± 2.12
Ic	1	Fed as Ib but after 3 days no supplement of NaCl was given but 5% glucose	↑ + 2-3%	28	46	60
	2			29	46	45
	3			37	53	90
	4			38	--	38
	5			36	58	50
Mean ± SD				34 ± 5*	51 ± 6**	57 ± 20*
Id	1	Fasting for 3 days	↓	16		16
	2			13		20
Mean ± SD				14 ± 2*		18 ± 3**

Control values	JGI Sprague-Dawley (10 rats)	JGI Wistar (6 rats)	G6PD MDI Sprague-Dawley (10 rats)	PRA (10 rats)
Mean ± SD	22.4 ± 4.49	25.1 ± 7	58.4 ± 8.4	19 ± 8.36

* "p" significant (<0.05) vs control group.
** "p" no significant (>0.05) vs control group.

TABLE 15

EXPERIMENT 1

GROUP II. Chronic sodium restriction.

Sub-groups	No of rats	Procedure	Duration	JGI	G6PD MDI	PRA
IIa	1	Fed Hartroft and Hartroft salt-deficient diet. (Sprague-Dawley rats).	1month	18	54	10
	2			30	59	70
	3			14	57	20
	4			34	66	12.8
	5			42	74	60
	6			26	79	20
Mean ± SD				27 ± 10	65 ± 10**	32 ± 26**
IIb	1	Fed NBC salt-deficient diet. (Sprague-Dawley rats).	1 month	20	45	20
	2			40	54	20
	3			14	107	20
	4			25	92	25
Mean ± SD				25 ± 11**	74 ± 30**	21 ± 2**
IIc	1	Fed corn salt-deficient diet. (Wistar rats).	1 month	34		
	2			32		
	3			48		
	4			44		
	5			37		
Mean ± SD				39 ± 7*		
IId	1	Fed NBC salt-deficient diet. (Sprague-Dawley rats).	2 months	40		50
	2			45		70
	3			82		140
	4			51		150
	5			42		50
	6			61		50
	7			29		18
Mean ± SD				50 ± 17*		75 ± 50*
IIe	1	Fed NBC salt-deficient diet. (Sprague-Dawley rats).	3 months	19		25
	2			48		75
	3			50		125
	4			33		40
	5			28		40
	6			40		120
Mean ± SD				36 ± 12*		71 ± 43*

* "p" significant (<0.05) vs control group.
** "p" no significant (>0.05) vs control group.

TABLE 16

EXPERIMENT 2
Chronic effects of NaCl and KCl on the JGI
MDI & PRA

Group	Sub-groups	No of rats	Procedure	Duration	JGI	G6PD MDI	PRA
I		1	1% NaCl	1 month	9	57	25
		2			12	54	0
		3			12	52	0
		4			12	70	19
		5			14	40	15
		6			16	68	15
		7			11		
		8			8		
		9			7		
		10			8		
		11			12		
		12			2		
	Mean ± SD				10 ± 4	57 ± 11	12 ± 10**
II	IIa	1	1% NaCl plus DCA	7 days	0		Traces
		2			13		
		3			3		
		4			14		
	Mean ± SD				7.5 ± 7*		
	IIb	1	1% NaCl plus DCA	21 days	0	4	
		2			0	3	no detect-able.
		3			0	13	
		4			1	9	
		5			0	5	
		6			0	5	
	Mean ± SD					6.5 ± 4*	
III	IIIa	1	2% KCl (Sprague-Dawley rats)	1 month	21		
		2			22		
		3			22		
		4			28		
		5			19		
	Mean ± SD				24 ± 8**		
	IIIb	1	2% KCl (Wistar rats)	1 month	22		
		2			28		
		3			26		
		4			21		
		5			39		
	Mean ± SD				27 ± 8**		

* "p" significant (<0.05) vs control ** "p" no significant (>0.05) vs control

Plasma renin activity : From 0.2 to 0.4 ml of venous blood was collected from the jugular vein of the rats under light nembutal anesthesia. Plasma renin activity (PRA) was measured by the method of Boucher et al. Before the onset of experiments 1 and 2, blood from 10 of these rats was taken for PRA as control values.

Body weights were measured every three days, and special care was taken about their nourishment and drinking during all the experimental period. Blood pressure was measured by tail plethysmography.

b) RESULTS.

The results are summarized in tables 14, 15 and 16.

Control values for the animals which received the standard laboratory diet and tap water "ad libitum" were the following : JGI 22,4 ± 4,9 for the Sprague-Dawley rats and 25,1 ± 7 for the Wistar rats ; MDI 58,4 ± 8,4 ; PRA 19 ± 8,36.

Experiment 1. "Sodium restriction".

Group I. Subacute sodium restriction for 3 days.

Subgroup Ia. The animals of this subgroup practically did not eat and on the third day they had lost about 8% of their initial body weight. The JGI (13,8 ± 7,8) was significantly decreased ($P < 0.01$). However, the PRA (16,7 ± 6) did not reflect a significant change ($P > 0.6$).

Subgroup Ib. No significant changes (JGI, $P > 0.6$; MDI, $P > 0.1$; PRA, $P > 0.3$) were observed.

Subgroup Ic. The animals of this subgroup contrary to the subgroup Ia at the end of the experiment gained about 2 to 3% of their initial body weight. A statistically significant increase ($P < 0.001$) in the JGI and PRA was observed. However, no significant changes ($P > 0.1$) occured in the MDI.

Subgroup Id. The two animals studied after 3 day of fasting showed a significant decrease ($P < 0.05$) in the JGI without significant changes ($P > 0.8$) in PRA values.

Group II. "Chronic sodium restriction". By rough estimate, the animals on the deficient diet ate only about one third as much as their controls after the third day of the experiment, their weight gain was slower than in the control animals.

Subgroup IIa. No statistically significant changes (JGI, $P > 0.2$; MDI, $P > 0.1$; PRA, $P > 0.1$) were observed after 1 month of sodium restriction using the Hartroft and Hartroft diet. However, relatively high values were noted in some animals in the three parameters studied.

Subgroup IIb. Similar findings to those observed in subgroup IIa were also encountered in this subgroup fed for 1 month with the NBC salt deficient diet (JGI, $P > 0.1$; MDI, $P > 0.1$; PRA, $P > 0.5$).

Subgroup IIc. A significant increase ($P < 0.02$) in the JGI ($39 \pm 6,78$) was encountered in this subgroup of Wistar rats fed corn salt deficient diet.

Subgroup IId. The JGI and PRA values were $50 \pm 17,2$ and $75,4 \pm 50$ respectively. Therefore, a statistically significant increase ($P < 0.001$ and $P < 0.02$ respectively) was observed after 2 months of sodium restriction.

Subgroup IIe. A significant increase in the JGI ($P < 0.01$) and in PRA ($P < 0.05$) were found.

In general the animals with the sodium deficient diet presented higher activity of the glucose-6-phosphate dehydrogenase in the proximal and distal tubules than the control animals.

Experiment 2.

Group I. The addition of 1% of saline as drinking fluid for 1 month to the 12 rats studied provoked a significant ($P < 0.001$) decrease of the JGI (10.2 ± 3.7). However, no statistically significant changes ($P > 0.1$) were observed in PRA (12.3 ± 10.2) in 6 animals studied, in spite of the fact that in two animals, the PRA was not detectable. No significant changes ($P > 0.7$) were observed in the MDI (56.8 ± 11).

Group II. The administration of DCA plus NaCl 1% resulted after the 7th day (Subgroup IIa) in a highly significant ($P < 0.001$) decrease in the JGI, and only traces of plasma renin activity were found. After 21 days (Subgroup IIb) a complete disappearance of juxtaglomerular granules, non detectable PRA and a highly significant ($P < 0.001$) decrease in the MDI were found. The blood pressure values in this group were 120 ± 6 mm Hg. The blood pressure of the control animals was on the average 115 mm Hg (85 to 135).

Group III. The addition of 2% of KCl as drinking fluid did not provoke a significant change ($P > 0.2$) in the JGI of Sprague-Dawley (Subgroup IIIa) or Wistar rats (Subgroup IIIb).

c) DISCUSSION.

The efficacy of the sodium-deficient diets employed was based on the absence of sodium in urine (598).

Our results show clearly the importance of the small amounts of the diet eaten by the animals. Thus the hypogranularity observed in such animals which did not eat the diet may not have derived from a degranulation process with subsequent release of renin since the PRA was in the normal range value.

We observed similar results when the animals were kept fasting. It is known that fasting decreases the formation of secretory material in many

secretory cells (586-588). We would like to suggest that in such experimental situations, there is a decreased formation of granules instead of a degranulation. This is reinforced by the observation that an increased JGI and PRA were observed in animals eating the diet (subgroup Ic). However, the MDI remained normal and this is consistent with previous observations (323, 326) that the glucose-6-phosphate dehydrogenase did not change significantly in rats submitted to a Na-deficient diet for short period.

Our results in rats maintained on chronic sodium restriction confirm earlier studies (32) demonstrating an increased JGI. However, we suspect that there are differences in responses from animals of different strains and from animal to animal of the same strain. Other factors must be considered : the amount of the diet eaten by the animals, the duration of the experiment and the adaptation of the animal to temperature changes or stress situations in the laboratory. Moreover, Fisher (199) did not find a significant increase in the JGI of adult rats after a month of sodium-deficient diet. Some of our animals also failed to show any significant increase in the JGI or in PRA after 1 month of sodium-deficient diet. The MDI was slightly but not significantly increased. Marx et al (326) have demonstrated that the G6PD activity of the macula densa of rats kept on a low sodium and high potassium diet, becomes slightly increased after 4 weeks and this activity became more marked in animals deficient for a longer time.

The significance of increased granularity of the JG cells in animals

submitted to low sodium diets has been discussed previously. Hartroft and Hartroft (32) suggested that the increased JGI probably represents a storage phase of secretion following a decrease in the rate of liberation of the granules. Later, Hartroft (53) and Tobian (203) suggested that the increased granularity might represent an augmentation of the secretory activity (renin secretion) of the JG cells. However, no direct evidence has been provided for any of these hypotheses. Our experiments with sodium deficient diets have demonstrated a correlation between the mean values of JGI and PRA. Furthermore, a parallel correlation was observed between the PRA activity measured weekly during the experiment (598) and the JGI at the end of it. These findings strongly suggest that the increase of JG cells granularity reflects a state of cell hyperactivity during stimulation with a resulting increase in renin synthesis storage and liberation, although storage seems to predominate over the releasing process.

The decrease of the JGI in rats submitted to high sodium intake experiments is accompanied by a slight but not significant decrease of the PRA. The Na^+ ion could possibly decrease renin synthesis or increase the intracellular destruction of the granules. Endes et al (600) have observed in rats, during the first 48 hours of high sodium intake, an increased granularity followed later by a decreased JGI.

An absence of correlation between JGI and MDI was observed in the group I of experiment 1. However, a significant decrease of MDI (exp. 2)

accompanied by disappearance of PRA and JG cells granularity was observed accompanied by an increase of G6PD activity in the distal tubule (except macula densa). The possible explanation of this finding will be discussed in the next section.

Our results on high potassium intake confirm the earlier studies of Hartroft (151) although no significant changes of PRA were demonstrated in our experiments.

In summary, our studies indicate that the JG cells granularity represents in rats with a low sodium diet or a high sodium load, functional cellular states of hyperactivity or hypoactivity respectively. The lack of correlation between the glucose-6-phosphate dehydrogenase activity of the macula densa (MDI) and the other parameters studied (JGI - PRA) was noted in various experimental situations.

B) THE JUXTAGLOMERULAR COMPLEX IN ADRENALECTOMIZED RATS, UNDER VARIOUS SODIUM INTAKES. ITS RELATIONSHIP WITH PLASMA RENIN ACTIVITY.

a) MATERIAL AND METHODS.

In this investigative section, more than 170 female rats weighing 180-220 g. of the Royal Victoria Hospital strain were used. Only data from rats living at the end of the experiments, will be presented. Bilateral adrenalectomy was performed by the lateral approach. The adrenals were removed with the capsule and surrounding adipose tissue. At the end of the experiment, the completeness of adrenalectomy was verified by autopsy.

The experiment was divided into two groups as indicated in the tables 17,18.

Group I: 45 adrenalectomized rats (T.17) were fed on the standard laboratory purina and tap water "ad libitum". Animals were sacrificed at the end of the first and second weeks.

Group II: 19 adrenalectomized rats were fed as in Group I, but received in addition 1% sodium chloride in their drinking water. The animals were sacrificed at the end of the first, second and third weeks (Table 18).

In addition, 13 intact female rats of the same strain and body weight served as controls. Their systolic blood pressure varied between 85 and 135 mm Hg.

Kidneys were removed, sectioned, fixed and stained as previously described. One half served for histological and juxtaglomerular granules staining (JGI), and, in some animals, the second half was used for histochemical studies (Glucose-6-phosphate dehydrogenase (G6PD), 6-phosphogluconic dehydrogenase (6PGD), triphosphopyridine nucleotide diaphorase (TPND) and α- glycerophosphate dehydrogenase (GPD) activity).

Plasma renin activity was also determined in some animals (Table 19) for possible relationship with the morphological findings.

b) RESULTS.

Results are summarized in tables 17, 18 and 19.

Adrenalectomized animals drinking tap water presented a gradual asthenia, loss of body weight (approximately 38% of the original values at the end of the second week) and low systolic blood pressures which, on the second week were on the average 56 mm Hg (52 to 60). In some animals it was very difficult to measure blood pressure by tail plethysmography pro-

TABLE 17

Group I (Adrex + tap water)

Number of rats	1 week duration		2 weeks duration	
	JGI	G6PD MDI	JGI	G6PD MDI
1	5		22	
2	19		16	
3	22		4	130
4	17		3	67
5	16		11	80
6	21		19	62
7	16		6	64
8	16	70	9	72
9	10	66	1	71
10	12	81	5	92
11	14	75	5	95
12	12	65	2	
13	37	105	7	
14	24	112	7	76
15	23	134	21	117
16	14	130	5	100
17	24	111	6	101
18	16		5	93
19	18		5	102
20	26		5	85
21	15		3	88
22	38		3	
23	15			
Mean ± SD	18.9 ± 7.69	94.9 ± 26.5	7.7 ± 6.1	87.9 ± 18.8
"p"*(vs control)	$p > 0.05$	$p < 0.001$	$p < 0.001$	$p < 0.001$

"JGI and MDI of adrenalectomized rats without sodium supplement."

* See control values in Table 19.

TABLE 18

GROUP II. (Adrex + NaCl 1%)

Number of rats	1 week duration		2 weeks duration	3 weeks duration	
	JGI	G6PD MDI	JGI	JGI	G6PD MDI
1	27	68	61	34	115
2	38	68	44	52	70
3	41	71	39	49	89
4	38	65	50	47	87
5	42	83	43		
6	51	93	26		
7			47		
8			46		
9			49		
Mean ± SD	39.5 ± 7.7	74.6 ± 10.9	45 ± 9.3	45.5 ± 7.9	90.2 ± 18.5
"p" vs control	<0.001	<0.01	<0.001	<0.001	<0.001

TABLE 19

Juxtaglomerular granular index (JGI), Plasma renin activity (PRA), Glucose-6-phosphate dehydrogenase in the macula densa (MDI) and ∝-glycerophosphate dehydrogenase (GPD) in adrenalectomized rats with or without NaCl supplement.

Group	Number of rats	Procedure	JGI	PRA	G6PD MDI	GPD (JGC)
I	1	Adrex + tap water (2 weeks)	7	440	76	3
	2		6	600	101	3
	3		3	500	88	2
	4		5	1250	93	1
	5		5	900	102	1
	6		5	1000	85	2
Mean ± SD			5.1 ± 1.3	782 ± 319	91 ± 9.1	2
II	1	1 week	27	50	68	2
	2	"	38	50	68	3
	3	3 weeks	52	25	70	1
	4		49	50	89	2
		Adrex + NaCl 1%				
Mean ± SD			41.5± 11.38	43.7 ± 12.25	73.7 ± 10.2	2
Control values Mean ± SD			(13 rats) 23.6 ± 6.71	(3 rats) 15.6 ± 5.85	(7 rats) 54 ± 6.8	(7 rats) 1

cedure.

The adrenalectomized rats which received 1% NaCl, as oral fluid intake, lost only 10-15% of their original weight at the end of the experiment. Their systolic blood pressures were on the average 84 mm Hg (77 to 92).

"Juxtaglomerular granulation index" (JGI)

The mean of the JGI of the control animals was 23,6 ± 6,77. In the rats of group I, (adrenalectomy + tap water), the JGI, after a week, was slightly but not signifcantly decreased (JGI 18,9 ± 7.69). The juxtaglomerular granulated cells were mostly of the type I. However, at the end of the second week, a high significative degranulation was observed (JGI 7,7 ± 6,1) ($P < 0.001$).

This degranulation was accompanied by an increased basophilia of the juxtaglomerular cells.

In group II, (adrenalectomy + 1% NaCl), a relatively inverse situation occurred as compared to the preceding group. A significant increase in JGI occurred after the first week (JGI = 39,5 ± 7,7) ($P < 0.001$) which was more marked in the following weeks (JGI = 45 ± 9,3 and 45,5 ± 7,9 in the second and third week respectively) ($P < 0.001$).

Histochemical results.

The mean of the MDI of the seven control animals was 54 ± 6,8. The average of α-glycerophosphate dehydrogenase activity at the glomerular vascular pole was 1 (weakly positive).

Group I. Adrenalectomy + tap water. An increased activity of the glucose-6-phosphate dehydrogenase activity of the macula densa expressed by the MDI was observed, both after the first and second week, following adrena-

lectomy. The MDI was 94.9 ± 26.5 and 87.9 ± 18.8. These values compared to those of the control animal were highly significant, ($P < 0.001$). A parallel increase was observed in the 6-phosphogluconic dehydrogenase activity of the macula densa. However, the increase of hexosemonophosphate shunt enzymes of the macula densa was not accompanied by a similar response in the triphosphopyridine nucleotide diaphorase. GPD at the vascular pole showed an increased activity as compared to the control animals.

Group II. Adrenalectomy +1% NaCl. The MDI was significantly higher in the first week (MDI = 74.6 ± 10.9) as compared to control animals, ($P < 0.01$). However, it was relatively lower than the MDI in the first week of Group I.

No significant difference was found in the MDI of group II at the end of the third week as compared to group I. However, a highly significant difference (MDI = 90.2 ± 18.5) ($P < 0.001$) was found as compared to the control group.

Plasma renin activity. (PRA)

The mean ± SE of PRA from these intact rats was 15.6 ± 5.85.

Group I. The highest value of PRA found in rats in the numerous studies done in our Institute corresponds to rat 4 (see table 19) reaching a level of 1.250 ng.

Group II. An increased PRA was observed in this group as compared to the control animals However, the values were much lower than in the Group I.

c) DISCUSSION.

The Royal Victoria Hospital strain was chosen because the rats lived longer after adrenalectomy without sodium supplement than those of the Sprague-Dawley strain.

From these experiments, it is clear that mild changes in sodium intakes profoundly affect the juxtaglomerular cells of the adrenalectomized rats. In Group I in which no sodium supplement was given, the cells become degranulated, whereas, in Group II in which sodium supplement was added, the juxtaglomerular cells became hypergranulated, Furthermore, the adrenalectomized animals of Group I, showed after 2 weeks very high values of PRA, accompanied by an increase in basophilia and GPD activity of the juxtaglomerular cells.

These findings show for the first time that hyperactivity of the juxtaglomerular cells can be associated with degranulated juxtaglomerular cells. It suggests that this degranulation represents a massive renin release, and constitutes further evidence for the secretory nature of the granular epithelioid cells and their relationship with renin production. On the other hand, we have shown, in the preceding section of this chapter and in the Group II of this experimental section, that an increase of PRA can occur in the presence of a juxtaglomerular cells hypergranularity, which is accompanied by an increased activity of GPD in the juxtaglomerular cells. (see table 19). From these findings, it appears that the granules represent increased synthesis and storage of renin. Renin may be released from the granules in amounts depending on the intensity of the stimulus acting on them.

The increase of the GPD at the vascular pole in Groups I and II, strongly suggests that in both degranulation and hypergranulation processes an active metabolic process occurs, possibly related to the synthesis of renin.

Several authors (16, 130, 214) have confirmed the observations of Dunihue et al (211, 213) who found an increased granularity of the juxtaglomerular cells in adrenalectomized rats. However, these investigators gave a substitution therapy to the animals or a sodium supplement. Our results in Group II fit well with these previous observations. On the other hand, Bohle and Tomsche (210) had reported that granularity and volume of juxtaglomerular cells were not modified by adrenalectomy in rats for a period of 10 days. However, the number of the cells appeared to be increased. Hennebert (28) has observed in adrenalectomized rabbits few granular juxtaglomerular cells during the first 24 hours following the adrenalectomy. Our findings in both groups therefore reconcile the findings of Dunihue and those of Bohle and Tomsche and Hennebert. In both cases, the cells seem metabolically hyperactive. It is possible that in adrenalectomized rats with a sodium supplement and slightly decreased blood pressure, the storage process predominates over the release process, a situation that may be also related to the rats maintained on a sodium-deficient diet.

Our findings in the hexosemonophosphate enzymes in the macula densa of adrenalectomized rats and triphosphopyridine nucleotide diaphorase activity provided further confirmation of the observations of Hess and Gross (323) in adrenalectomized rats after a period of a week.

Since groups I and II presented in spite of their different JGI an increased activity of the G6PD and 6PGD in the macula densa, no quantitative relationship was found between the PRA and MDI values in Group I as compared to those found in Group II. It is suggested, as a working hypothesis, that

the role of the macula densa enzymes in adrenalectomized rats might well be related to sodium balance, acting as an energy-yielding process in sodium reabsorption in order to compensate the absence of the mineralocorticoid hormones.

CHAPTER IV

The two main purposes of this study were : a) to look for any morphological changes in the macula densa in sodium-restricted rats with a high JGI.

b) To find out if the juxtaglomerular granulated cells would be derived from the arteriolar smooth muscle since myofilaments have been described in these epithelioid cells.

A) ELECTRON MICROSCOPIC STUDIES OF THE JUXTAGLOMERULAR COMPLEX OF NORMAL AND SODIUM RESTRICTED RATS.

a) MATERIAL AND METHODS.

Kidney tissues from control and sodium restricted Wistar rats were used. The fixation was accomplished by dripping cold, isotonic buffered osmium tetroxide 2% on the surface of the left kidney in the living animal. Osmium-fixed blocks were embedded in Epon after dehydration in alcohol, serial thick and thin section were cut as described in the chapter I and stained by Reynold's method (503).

b) RESULTS.

1) Juxtaglomerular granulated cells.

Electron micrographs of the juxtaglomerular granulated cells of sodium restricted rats show an increase in granularity and size of these cells. Some of these cells were so filled with granules that the ground substance of the cytoplasm could hardly be seen (Fig. 37).

Plate X to XXIV. "In vivo". Osmium tetroxide fixation.

Plate XXVI to XXX "In vivo" glutaraldehyde fixation (see text)

All sections were stained with Reynolds' method. (503)

PLATE X

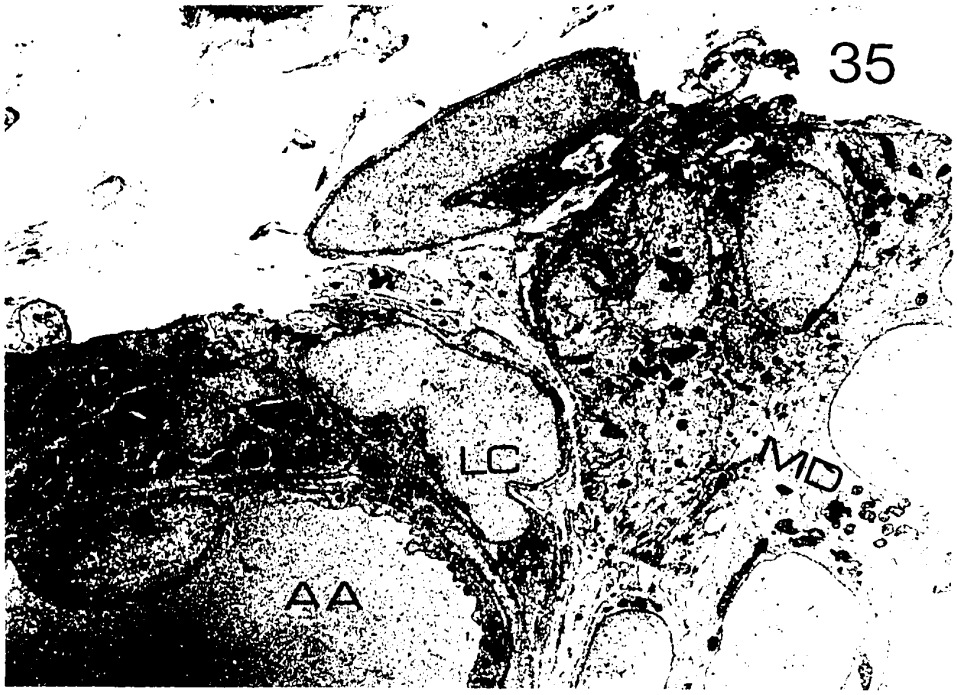
FIG. 35. Kidney from a control rat. Afferent arteriole (AA). Granular cells (GC). Lacis cells (LC). Macula densa (MD) (cut obliquely). x 4 250.

FIG. 36. High magnification of a serial cut from the upper left portion of fig. 35. Granules revealing a fine granular content (G). Note (Arrow) the space within the endoplasmic reticulum which is continuous over the adjacent granule. Mitochondria in the center of the field. Myofilaments (My). x 50 000.

PLATE XI

FIG. 37. Oblique section of an afferent arteriole showing the endothelium (Ed). Smooth muscle cell (SM). Large granular cell (GC). Distal tubule (DT). Rat which received a sodium deficient diet for 1 month. x 7 500.

FIG. 38. Higher magnification of a granular epithelioid cell from fig.37 with a well developed Golgi apparatus (Go), (see text). Multivesicular bodies (Arrow), some with dense material (Double arrow) and others with myelin-like figures (Large arrow). Granules (G). Mitochondria (M). Myofilaments (My). Nucleus (N). Distal tubule (DT). x 12 500.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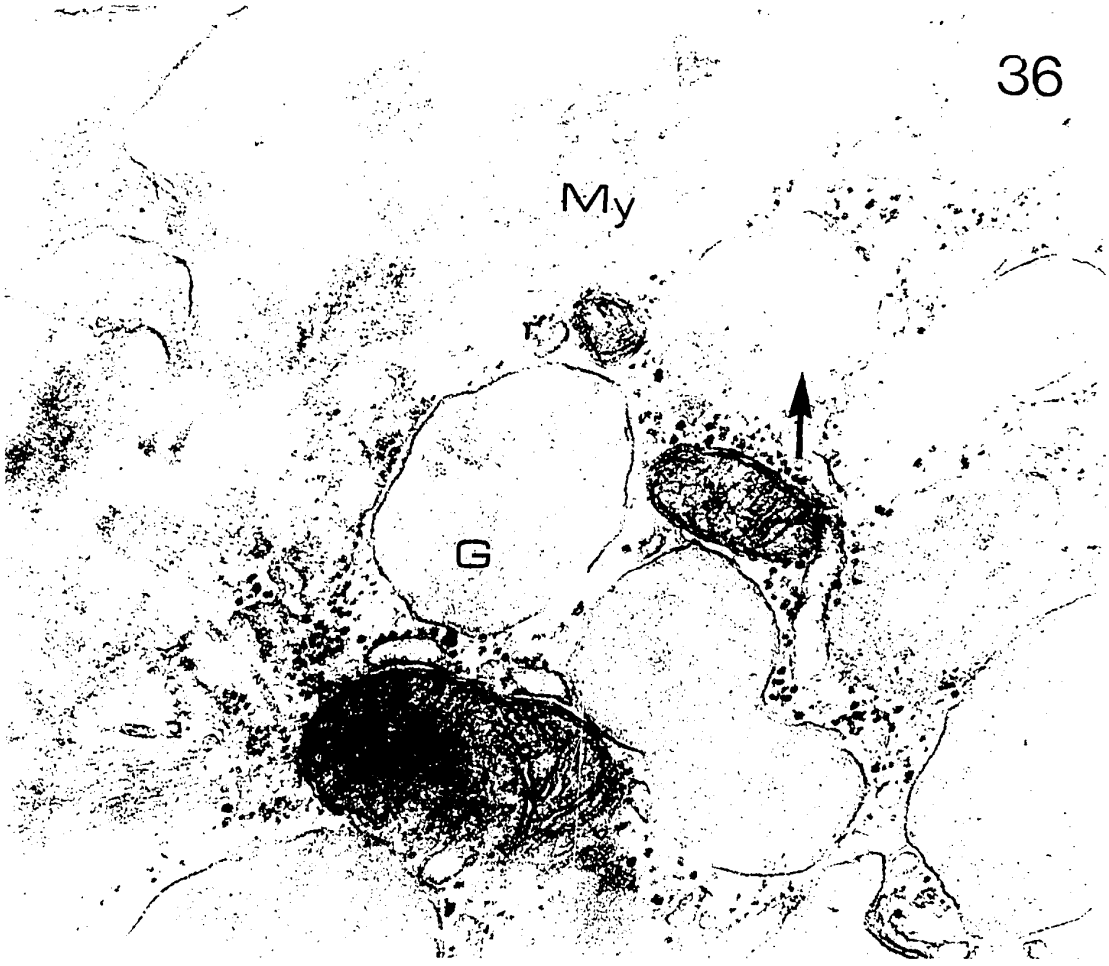
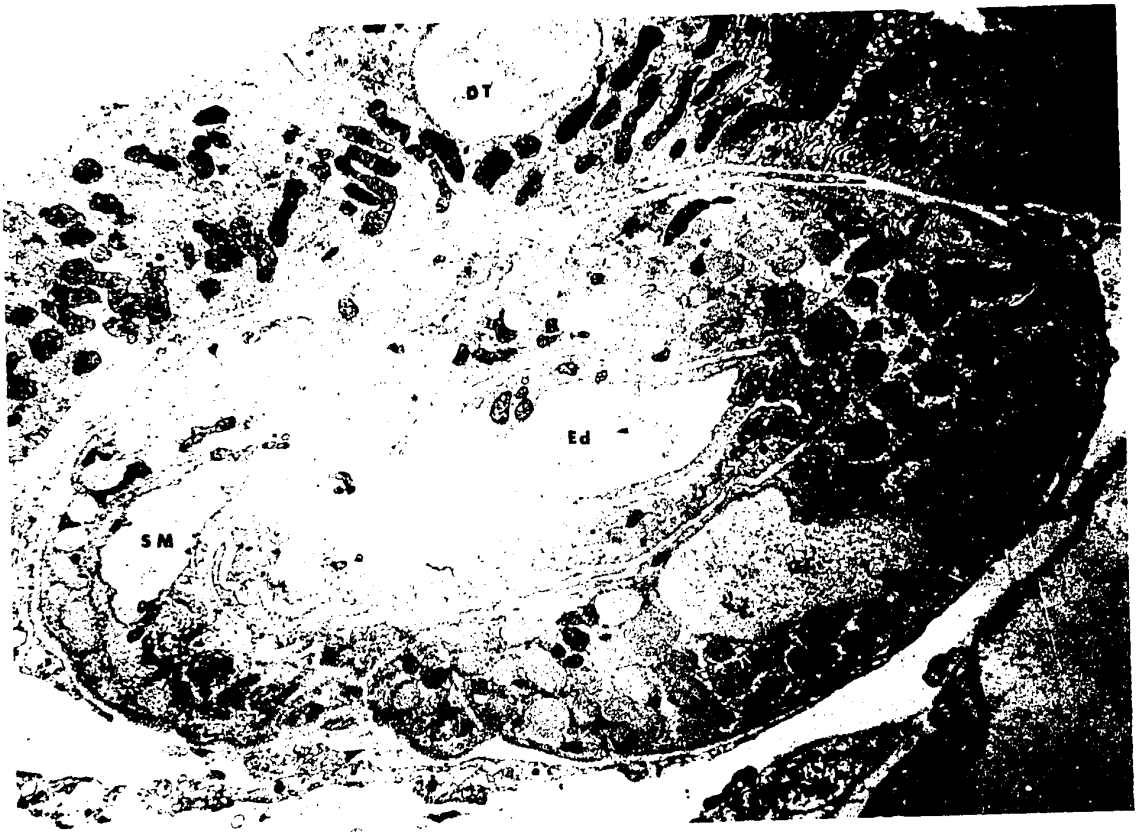
LC
MD
AA


36
My
G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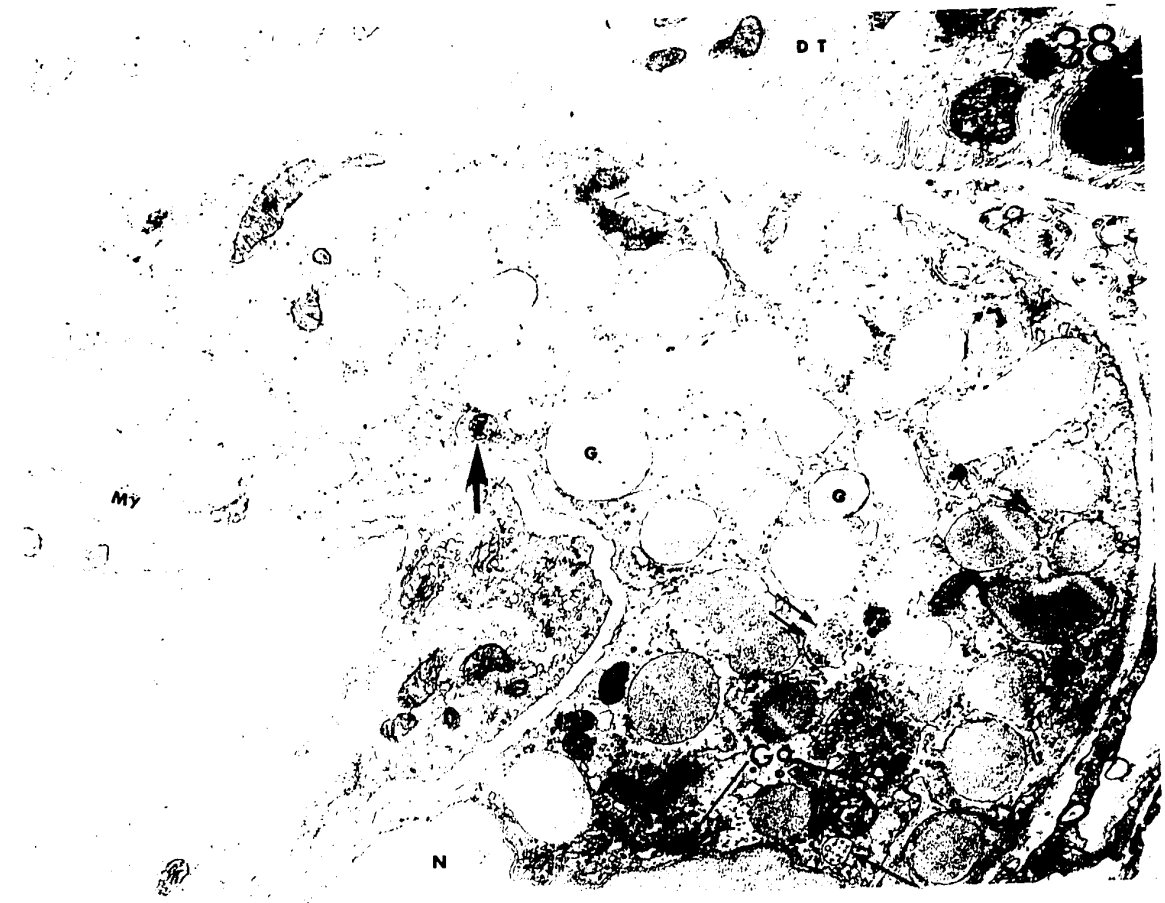
DT
38
My
G
G
G
N

Granules.

The granules of juxtaglomerular cells in both normal and sodium restricted rats varied considerably in size from about 0.1 to 1 μ or more (Fig. 36, 38). The granules had either a circular or an oval profile. Occasionally, a few granules appeared to coalesce to form larger bodies. The granules contain relatively homogenous material of moderate density. Sometimes, the contents were more or less flocular (Fig. 38), revealing in some regions the presence of fine granules or filaments from approximately 100 to 200 A^{o} in diameter (Fig. 36). In sodium-restricted rats some granules contain denser material or myelin-like figures resembling lysosomes forms (Fig. 38, 40). Multivesicular bodies were often seen near the Golgi zone, occasionally with moderately dense material content (Fig. 38). Granules, as shown in fig. 40 may not be completely delimited by membranes. Where granules showed a membrane discontinuity, microspheres from about 200-300 A^{o} were present (Fig. 40). These microspheres seemed more numerous in granules located at the cellular periphery. Figures 34 and 40 reveal the presence of microspheres in the vicinity of the granulated cell membrane, showing that they are smaller than micropinocytic vesicles. No granular extrusion through the cell membrane was observed.

Nuclei.

Nuclei of juxtaglomerular granulated cells were ovoid in shape and sometimes indented. In sodium-restricted rats, the nuclei of granular cells seemed to be larger than in control rats. Prominent nucleoli could be seen in some favorable sections.

Golgi apparatus.

In sodium-restricted rats, the Golgi apparatus of the juxtaglomerular

PLATE XII

FIG. 39. Topographic view of the juxtaglomerular apparatus showing the macula densa (MD) in which the absence of basal infolding membranes, the presence of sub-basilar extracellular compartments can be observed. Lacis cells (LC). Afferent arteriole (AA) entering the glomerulus (GL). Endothelium (Ed). Several granular cells (GC). No preglomerular sphincter is visualized. (Rat which received a sodium deficient diet for 1 month).
x 4 250.

PLATE XIII

FIG. 40. Enlarged field of a granular cell from the lower region of fig. 39 showing granules with different densities and forms. Some of the granules seem to be limited by an incomplete membrane and showing in the vicinity of its discontinuity, zones with microspheres (Arrows). Myofilaments (My). Lysosome-like form (Ly). Endothelium (Ed). x 32 500.

FIG. 41. Enlarged area of a serial section of the cell at the lower left region of fig. 39, showing microvesicles flattened and large vesicles in the Golgi apparatus (Go). Nucleus (N). A large granule on the upper right of the field. Mitochondria (M).
x 50 000.

PLATE XII

FIG. 39. Topographic view of the juxtaglomerular apparatus showing the macula densa (MD) in which the absence of basal infolding membranes, the presence of sub-basilar extracellular compartments can be observed. Lacis cells (LC). Afferent arteriole (AA) entering the glomerulus (GL). Endothelium (Ed). Several granular cells (GC). No preglomerular sphincter is visualized. (Rat which received a sodium deficient diet for 1 month).
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x 50 000.

PLATE XII

PLATE XLI

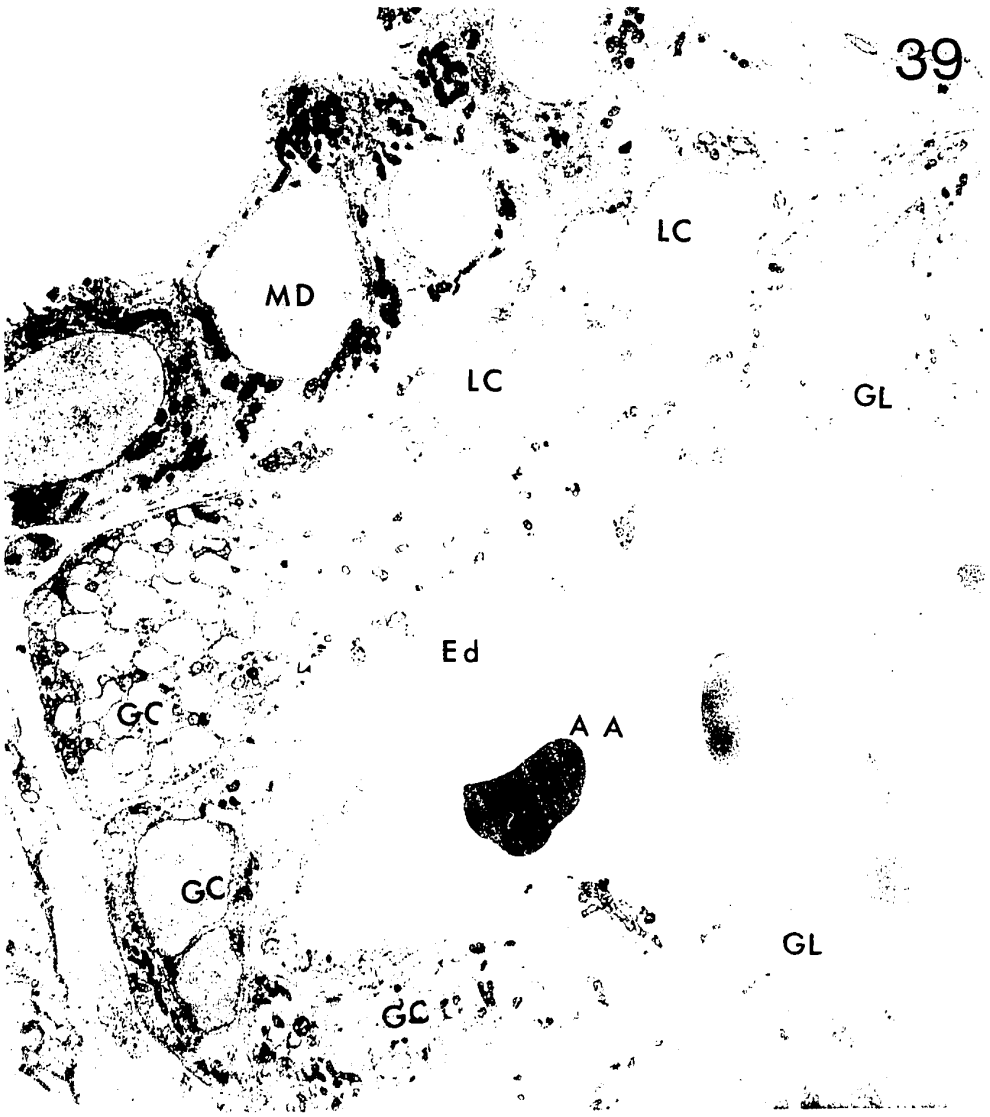


PLATE XIII

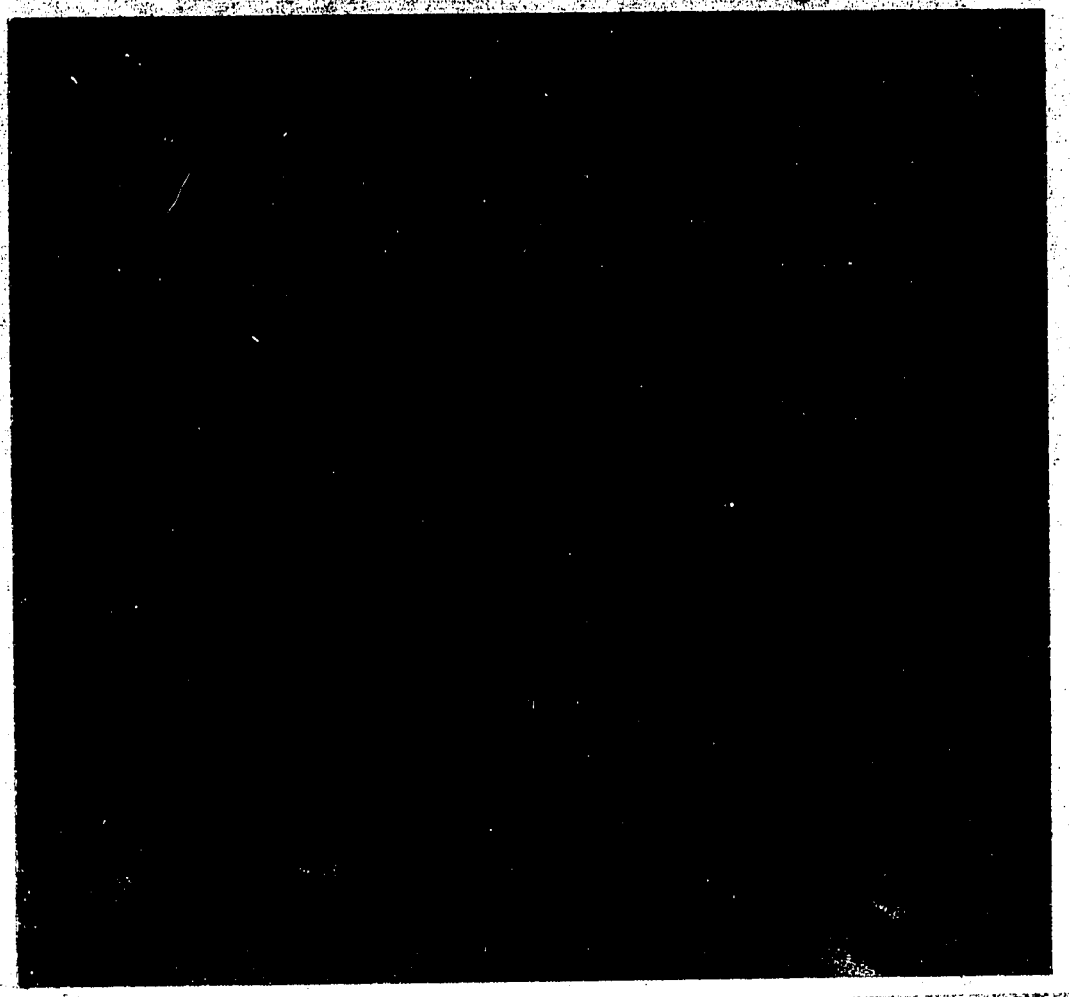
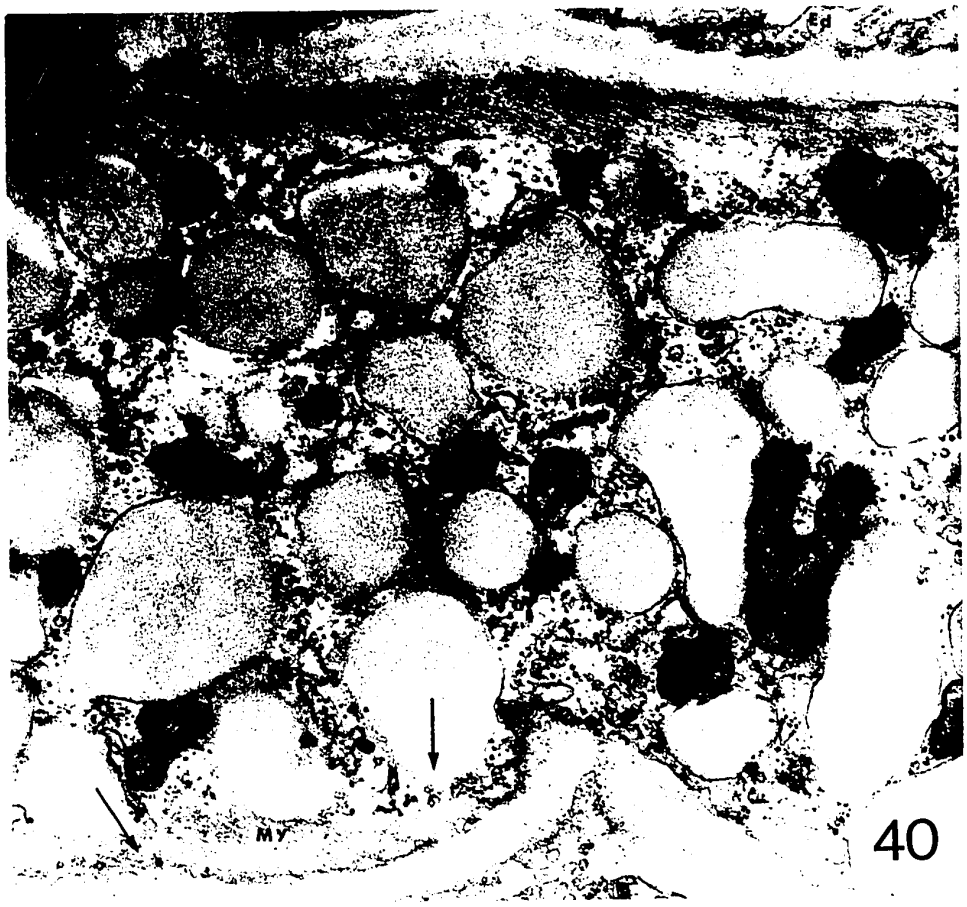
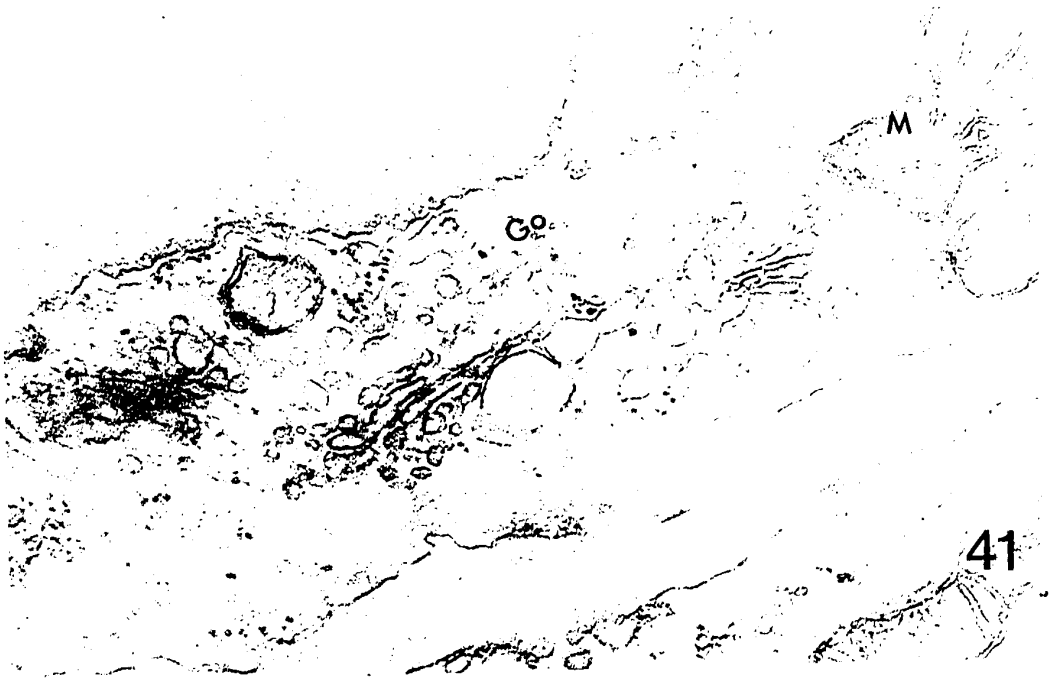


PLATE XIII



40

N



41

granulated cells consists of flattened vesicles, micro-vesicles, and large vesicles. These three components of the Golgi zone contain dense material having the same electron density as the granules described above. (Fig. 38, 41).

Endoplasmic reticulum.

A moderate variation in numbers of ribosome particles may be found in the granular cells. The reticulum generally showed dilatations and its cisternae contained dense material consisting of small granules or filaments similar to those described above within the JG granules. This material is more apparent in sodium-restricted rats (Fig. 38). At one stage of the granule formation, the plane of section passed through distended endoplasmic reticulum where it was directly continuous with the limiting membrane of a granule or where its contents were confluent with the matrix of a granule (Fig. 36). Sometimes dense material of the same electron density as the granule matrix were found limited by ribosomes (Fig. 38).

Other cytoplasm components.

Microtubule-like formations were found in control and sodium-restricted rats. Bundles of myofilaments were often seen in control as well as in sodium-restricted rats (Fig. 36, 40). Mitochondria seem to be more numerous in the sodium-restricted than in the control rats.

2) Lacis cells.

In control and sodium-restricted rats, lacis cells did not show any morphological differences, nor any changes in the cell size. The nuclei showed an ovoid, elongated or indented shape. Prominent nucleoli could be seen in some sections. Lacis cells could be found in the walls of

arteriole. They did not contain granules resembling those of granular cells. However, some irregular dense structures resembling lysosome were occasionally found (Fig. 35). The Golgi apparatus, when visualized, was well developed and consisted of flattened and small vesicles. The endoplasmic reticulum was less extensive than that seen in the granular cells and the ribosomes were generally fewer, giving the cytoplasm a less dense appearance. The cytoplasm contained a few to moderate number of mitochondria. Occasionally, bundles of fine fibrils were found in the cytoplasm with areas of increased density, specially near the plasma membrane. The lacis cells were usually separated between them and also from the macula densa and juxtaglomerular cells by an intercellular substance or matrix material with a density like that of basement membranes (Fig. 44), giving the morphological aspect resembling a conjunctive network, as it was described by Oberling and Hatt (14). The cells had a number of processes, both large and small. No zones of discontinuity in this intercellular substance in the contact zones with macula densa cells or juxtaglomerular cells were observed. Evident transitional forms of lacis cells to granular cells were not noted. Neither blood, nor lymphatic capillaries were found among the lacis cells.

3) Macula densa.

The macula densa cells showed some features appearing also with light microscopy. The nuclei lied closer together than in other parts of the tubule and fewer infolding membranes-mitochondria complex were noted. With electron microscopy, further distinguishing features become more appearent.

Dense bodies.

Occasionally, a few dense irregular bodies resembling lysosome-forms

PLATE XIV

FIG. 42. Higher magnification of a serial cut from the lower right portion of the macula densa of fig. 35. Mitochondria (M). Dense bodies (Short arrow). Note the parabasal localization of the Golgi apparatus (Go). Nucleus (N). Microvilli (large arrow). Basement membrane (Small arrow). Lacis cell at the right of the small arrow. Note the presence of extracellular compartments at the base of the cell and the absence of infolding membranes. Control rat. x 17 500.

FIG. 43. Higher magnification from the middle part of the macula densa of fig. 39. Tubular lumen (Lu). Note the prominent and lateral localization of the Golgi apparatus (Go). Extracellular compartments (Arrow). Rat which received a low salt diet for 1 month. x 17 500.

PLATE XV

FIG. 44. Higher magnification of a serial section of fig. 39. Macula densa (MD). Golgi apparatus (Large arrow). The macula densa basement membrane appears very thin in some places (Small arrow). Lacis cells (LC). Granular cell (GC). x 7 500.

FIG. 45. High magnification of a basal part of a macula densa (serial section of fig. 39) from a rat which received a low salt diet. Note the communication of the extracellular compartments with the sub-basement membrane space (Arrow). Nuclei (N). Mitochondria (M). Basement membrane (bm). Lacis cell (LC). x 50 000.

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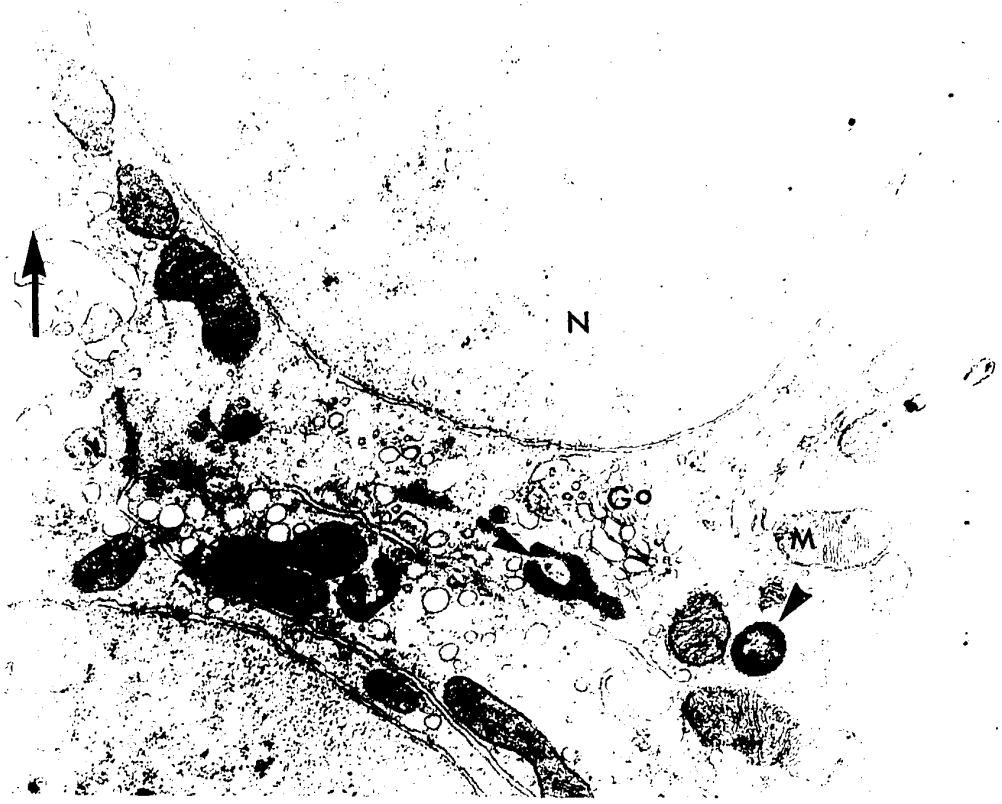

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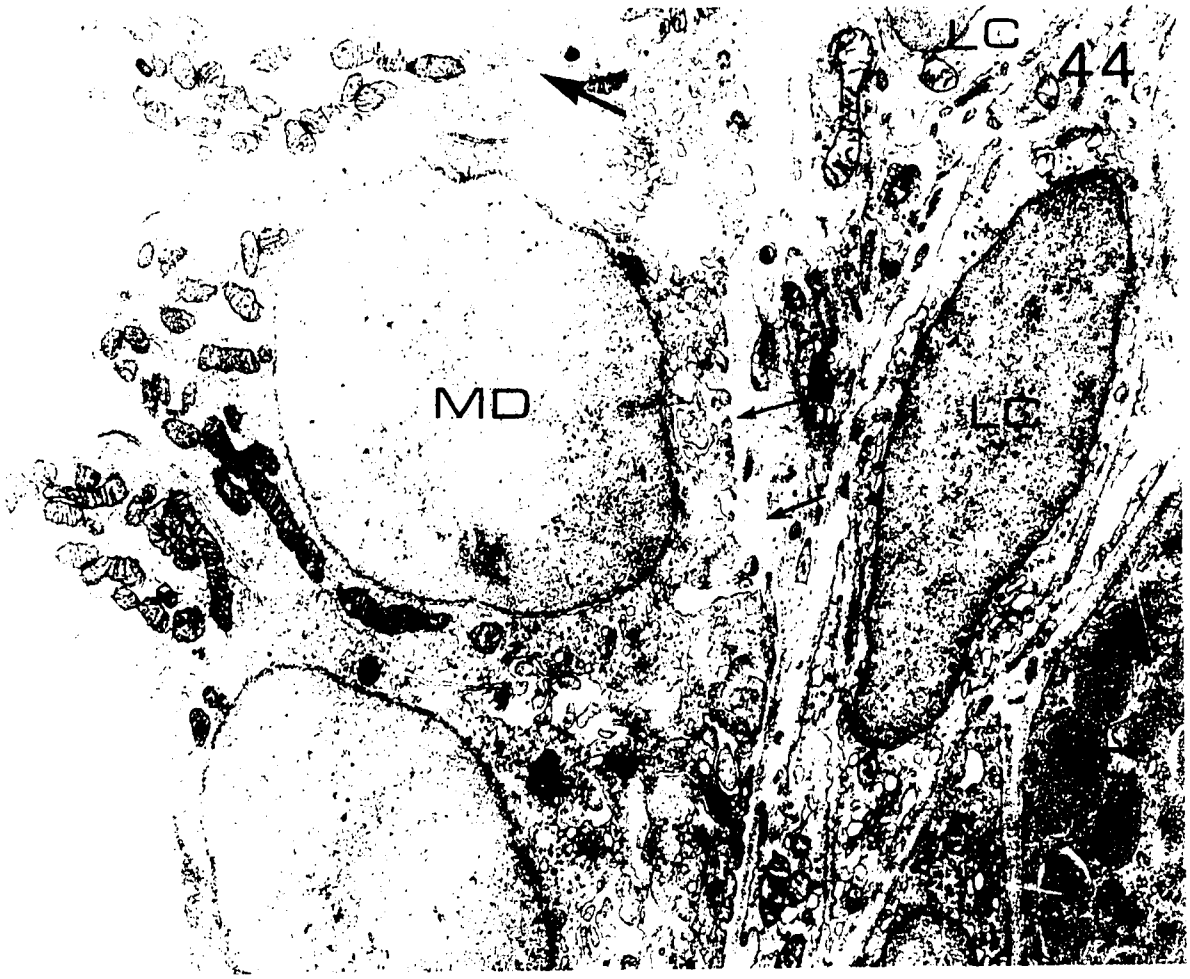
LC
44
MD
LC


LC
bm
45
N

were found in the cytoplasm of both control and sodium-restricted rat (Fig. 42, 43). However, no granules similar to those found in juxtaglomerular cells were observed.

Golgi apparatus.

The location of the Golgi apparatus in the macula densa cells seems to be variable. Most often, as especially in control rats, it was located in the basal or parabasal region (Fig. 42). Sometimes, it was seen in the latero-medial region. It consisted of flattened vesicles and micro-vesicles. In sodium-restricted rats, the Golgi apparatus was large and prominent (Fig. 43). Its location was mostly lateral to the nuclei. No dense material was observed in any of its components.

Endoplasmic reticulum.

It was composed of relatively small amounts of endoplasmic reticulum, and a moderate number of free ribosomes. It was difficult to visualize any qualitative or quantitative difference between control and sodium-restricted rats.

Mitochondria.

The mitochondria are less numerous, shorter, thinner and irregularly distributed when compared to the rest of the intermediary segment. In some sections, there seems to be an increased number of mitochondria in the macula densa cells of the sodium-restricted rats.

Cell surface.

Microvilli were often found on the luminal surface. Functional complexes were encountered more or less regularly.

The smooth outline of the tubule became irregular over the basal surface, where the processes of the Lacis cells impinged upon it. The

infolded membranes at the base of the cell were fewer and irregularly arranged without having a close relationship to the mitochondria as observed in other parts of the distal tubules. The plasma membranes separate at the base, sometimes forming extracellular or sub-basilar compartments. No marked differences in the cell surfaces of the control and sodium-restricted rats were observed, save for more numerous sub-basilar compartments in sodium-restricted rats (Figs. 43, 45).

Basement membrane.

The basement membrane was found to be thinner than in other parts of the distal tubule. No interruption or discontinuities were found at any place, in the control or the sodium-restricted rats. In some sections, it was difficult to distinguish it from the intercellular matrix material.

c) DISCUSSION.

The electron microscope studies of JG cells in normal and sodium-restricted rats indicate formation of at least some of the granules in the cells. The similarity between the electron opacity and granularity of the material within the cisternae of the endoplasmic reticulum and that of the JG granules suggests, that the material (possibly renin) in the granules is synthesized within or by the elements of the reticulum. Direct transfer from within endoplasmic reticulum to some granules appears to occur in fig. 36. In other protein secreting cells it has been shown that the endoplasmic reticulum is the site of synthesis of the protein concerned. The different sizes of granules and the appearance of dense material at the Golgi region also suggest that the storage of the synthesized material and the maturation of the granules may also take place there. According to previous reports, a cycle

of synthesis in the juxtaglomerular cells of the rat is suggested following the general pattern observed in other secretory cells (87-98).

Thus, the secretory material would be synthesized on the ribosome template in the endoplasmic reticulum and conveyed to the Golgi apparatus where it accumulates in vesicles until it eventually becomes separated and appears in the cytoplasm as a secretory granule limited by a single unit membrane (92-97). However, the release of the secretory material appears to be different from other secretory cells. Although we have studied numerous and serial thin sections, we have not observed in any instance a fusion of the granule membrane with the cell membrane suggesting an extrusion of secretory material. As far as we know, no such finding has been reported in the literature concerning the granule secretion of the JG cells. Lee et al (68) have reported in experiments involving hypertonic peritoneal dialysis and subcutaneous administration of cobaltous nitrate injections in others, a degranulation followed by a regranulation process. During the degranulation state (mostly in cobalt treated rats), they observed occasionally in the intercellular spaces an electron dense material resembling the granule content. PRA was not measured. On the other hand, one should not forget the cytotoxic effects of the cobalt salts described by Van Campenhout (101), and Hennebert (100), at even much lower doses than that employed by Lee et al. Furthermore, no significant changes in the JGI of rat kidneys were noted after peritoneal dialysis (199).

We do not know how the secretion of the granules occurs, but it appears to be different from other secretory cells. Our demonstration of a layer of myofilaments at the periphery of the JG cells suggests that the fusion

of the granule membrane with the cell membrane would not occur, on account of this barrier of myofilaments at the inner surface of the cell. A possibility is that the granule membrane could open and release its content. Then the released material will go through the myofilaments to gain the cell membrane. The presence of granules which were not completely delimited by membranes (mostly at the periphery of the cell), showing microspheres inside and outside of the granules favors such possibility (See fig. 40). These microspheres would then represent the release of the biologically active molecule, which would pass through the barrier or between the myofilaments (see fig. 40). An unlikely alternate possibility is that such microspheres might represent some precursor of the granules brought from arterial blood through the cell surface to serve in the synthesis of the definitive biologically active molecule through mediation of the endoplasmic reticulum of the JG cells.

Hess and Regoli (157), Chandra et al (62), and Dyrda (252) have proposed that renin or its precursor, instead of being formed within the JG cells, could be synthesized in the macula densa and then taken up by the JG cells and then this substance will be stored in such cells, where the maturation of the granules could take place.

Our observation of the macula densa cells does not support this theory. No dense material similar to that of the JG granules, microspheres, or micropinocytic vesicles in the macula densa cells were observed. However, in sodium-restricted rats, the relatively enlarged Golgi apparatus, its lateral localization in some cells, and the presence of large intercellular compartments suggest that the macula densa might well act in some reabsorption process of the tubular fluid. It is difficult to say whether or not the

number of mitochondria is increased in sodium-deficient rats when compared to the normal animals. However, the number of mitochondria encountered in normal and sodium-restricted animals does not correspond to the activity of the G6PD observed in such macula densa. Dense bodies, resembling those described by Barajas and Latta (16) in adrenalectomized rats were observed in both control and sodium-restricted rats, without quantitative differences. Moreover, such dense bodies were also found in other parts of the distal tubule.

Since the lacis cells did not present transitional forms of granular cells, either in control or in sodium-restricted animals, we suggest that the increased granularity in sodium-restricted rats depends on the increase of the granular cell content and on the transformation or metaplasia of the arteriolar smooth muscle cells into granular cells. This point of view will be demonstrated in the next section of this chapter.

The increase in number of granules in sodium-restricted rats, accompanied by relatively dilated endoplasmic reticulum and prominent Golgi apparatus strongly suggests that synthesis of secretory material is increased. Chandra et al (62) reported that the pressor material (renin) corresponds to the fine granules contained within the JG granules, and suggested that the contents of these granules are either only stored in the JG cells during the period of sodium depletion, or the rate of discharge is less than the rate of synthesis. As discussed in relation to previous experiments in sodium-deficient rats (Chapter III), it is suggested that the synthesis, the storage and the release of the renin are increased with the storage process predominating over the release process.

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An observation that might well implicate a functional significance is the absence of a sphincter-like formation at the entrance of the afferent glomerular arteriole in the glomerulus. Clara (599) and De Muylder (282) in light microscopic studies claimed to observe such a sphincter. However, fig. 39 clearly demonstrates that the smooth muscle cells are replaced by granular cells, which appear to have on the basis of their large size, a relatively smaller concentration of myofilaments. It suggests that the role of the granular cells is more of a secretory than a contractile nature. It is difficult to explain the experiments of Thurau et al (378-381) on the basis that angiotensin formed locally in the site of the JG cells could constrict the afferent arteriole at this place. However, any decrease in number of myofilaments may be only relative since the whole cell is enlarged and therefore one would expect great diminution of myofilaments in any of one section even if the total number remained similar to that of the medial muscle cells.

B) THE JUXTAGLOMERULAR CELLS OF RATS TREATED WITH MERCURY BICHLORIDE. THE DIFFERENTIATION OF GRANULAR EPITHELIOID CELLS FROM ARTERIOLAR SMOOTH MUSCLE CELLS. LIGHT AND ELECTRON MICROSCOPIC STUDIES.

a) MATERIAL AND METHODS.

Thirty-eight Wistar rats of both sexes, with a mean initial body weight of 190 g. (range 170-200 g.), were subdivided into 2 groups.
Group 1 (15 rats) were used as controls.
Group 2 (23 rats) received a single intramuscular injection of mercury bichloride ($HgCl_2$) at a dose of 12 mg/kg in a 0.5 ml isotonic saline serum. Then 5, 3 and 15 rats were sacrificed 16, 40 and 72 hours later respectively. One kidney was used for light microscopy, using the fixation procedures and stains already described for determination of JGI. The other

kidney was used for electron microscopy using the osmium tetroxide fixation. Thin serial sections were stained with Reymold's method.

b) RESULTS.

Juxtaglomerular indices of granularity.

Table 20 illustrates the changes in the mercuric bichloride treated rats during the experimental period of 72 hours. The result of the JGI mercuric bichloride treated animals showed that, whereas it remained nearly the same as in the control group during the first 40 hours, a significant increase occured 72 hours following injection (JGI 42.4 ± 9.3) ($P < 0.001$).

Electron microscopy. Juxtaglomerular granulated cell differentiation.

Fig. 46 (rat killed 72 hours after $HgCl_2$ injection) shows a longitudinal section through an afferent arteriole before entering the glomerulus. This arteriole reveals the presence of various morphological types of cells in the media. An arbitrary classification of these cells was made according to their amount of myofilaments and granules. a) Smooth muscle cells (SM) (Fig.s 46-48) in which only myofilaments were visualized, but no granules. b) Transitional granulated cells (TG_{1-4} Fig. 46) in which a large amount of myofilaments and relatively few granules were observed. TG_1 and TG_2 (Figs. 47-49) possibly represented early transitional granulated cells. c) Granular epithelioid cells (GE) (Figs. 46, 52) in which the granules were so numerous that they almost completely fill the cell cytoplasm with only some bundles of myofilaments at the cell periphery. The nuclei of the transitional granulated cells were large, ovoid or indented in shape (TG_1,TG_2,TG_3). The cells appeared to become enlarged, thus adopting an epithelioid aspect.

TABLE 20

JG cell granularity in mercury bichloride treated rats.

Number of rats	Group I (control) JGI	Group II ($HgCl_2$)		
		(+ 16 hr)	(+ 40 hr)	(+ 72 hr)
1	29	18	35	40
2	26	20	26	46
3	18	23	17	47
4	29	22		31
5	31	18		57
6	17			59
7	28			41
8	23			50
9	20			46
10	24			42
11	27			35
12	17			26
13	18			30
14	24			44
15	22			43
Mean ± SD	23.5 ± 4.7	20.2 ± 2.29	26 ± 9	42.4 ± 9.3*

* "p" (vs control)= < 0.001

PLATE XVI

FIG. 46. Rat treated with $HgCl_2$ (+ 72 hours). Tangential section through the course of an afferent arteriole before it enters the glomerulus. In this section, three morphologically different cell aspects in the muscularis are shown. Smooth muscle cells (SM). Transitional granulated cells ($TG_{1,2,3,4}$) and granular cells (GC). Endothelium (Ed). Arteriolar lumen (Lu). x 4 250.

PLATE XVII

FIG. 47. Serial section of TG_1 showing the enlarged nucleus (N). Golgi zone (Go). Lysosome-like forms (Ly). Granule (G). Myofilaments (My). Endothelium (Ed). Micropinocytic vesicles (Arrow). x 17 500.

FIG. 48. Enlarged area of a serial section from fig. 46, comparing a smooth muscle cell (at the left of the field) with a transitional granulated cell (at the right). Nucleus (N). Golgi apparatus (Go). Myofilaments (My). Dense bodies (Large arrow). Micropinocytic vesicles (Small arrows). Granules (G). Nerve terminal areas (NA). Endothelium (Ed). x 17 500.

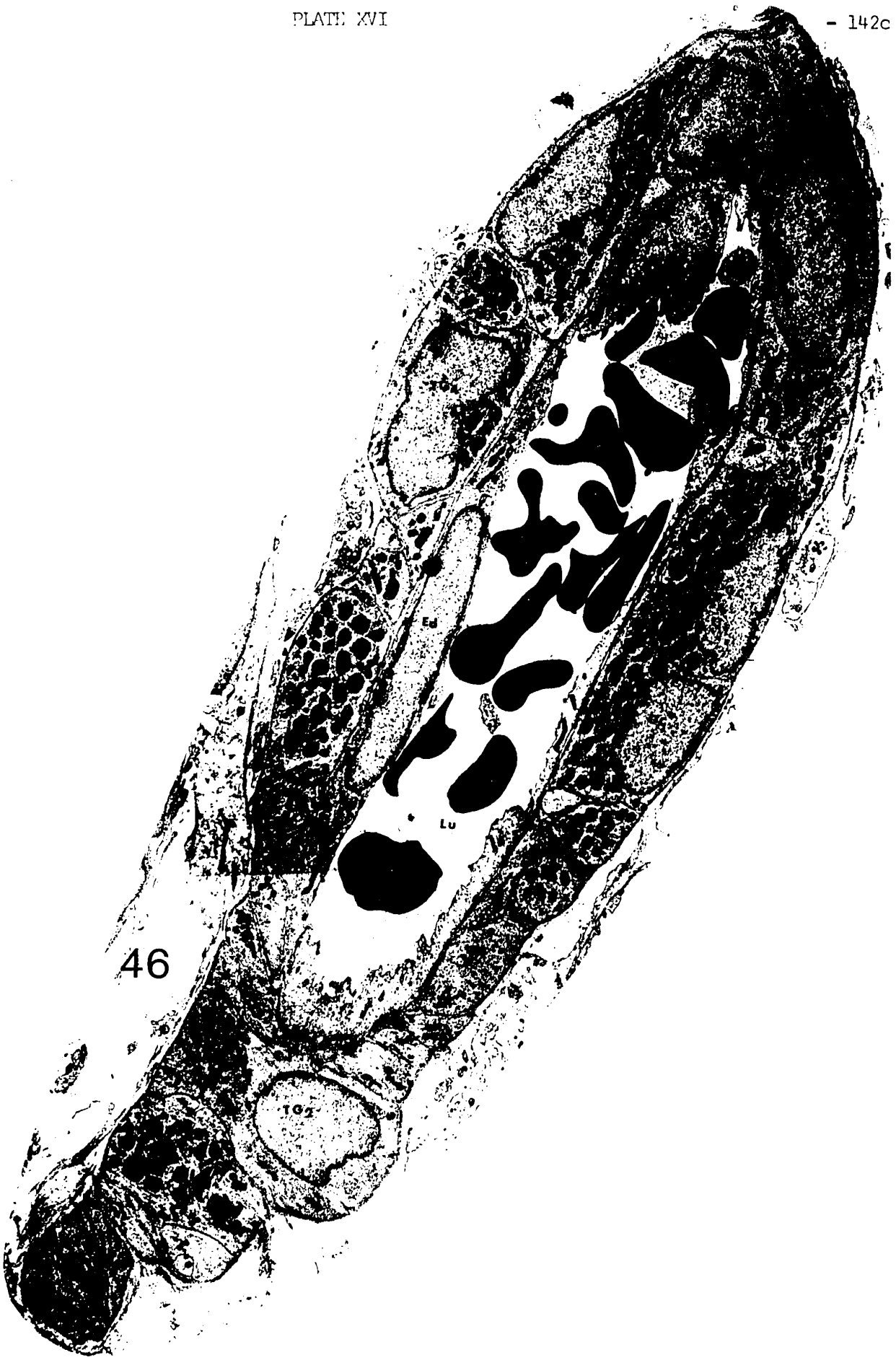
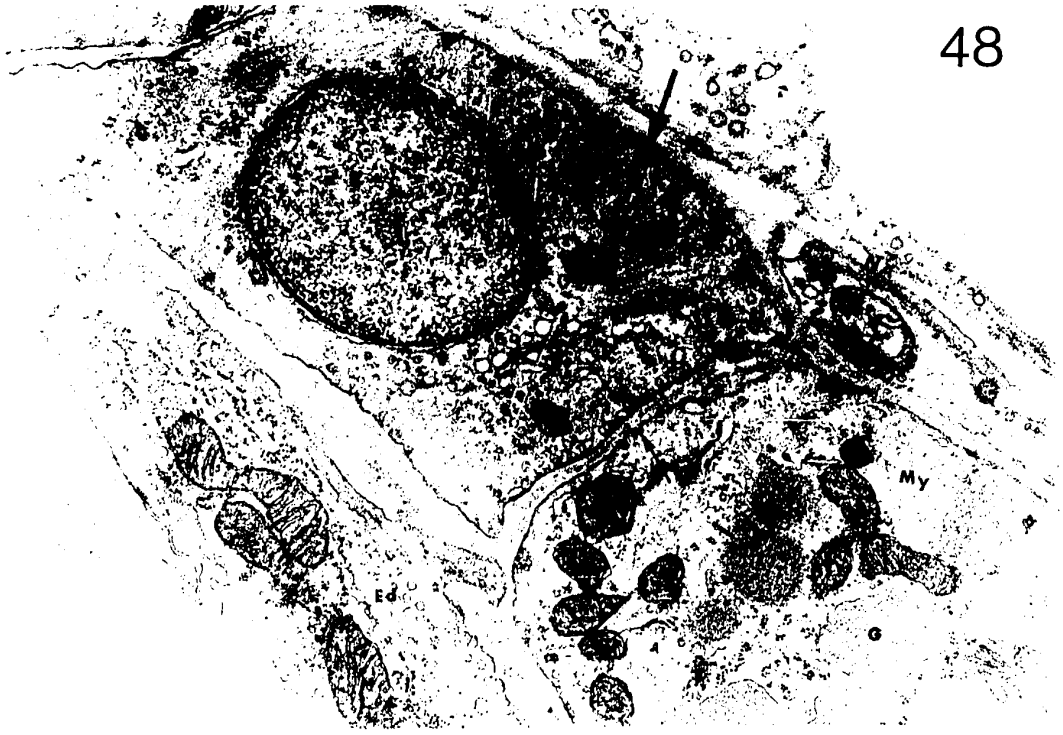

Ed
Lu
TG2
46

PLATE XVII

PLATE XXII



The number of free ribosomes seemed to be increased as well as the endoplasmic reticulum (Fig. 50).

In these transitional cells the number of granules varied from few to moderate. Some granules appear near the Golgi zone (Fig. 51). Some of them present lysosome-like forms. The general features of the granules were the same as described in the previous section of this chapter. The Golgi apparatus presents a considerable number of microvesicles (Figs. 49, 51). In some sections, the mitochondria seemed to accumulate in the neighbourhood of the newly differentiated zone and their number were moderately increased (Fig. 49 TG_2). The myofilaments, dense bodies and micropinocytic vesicles were numerous. Some vesicles from 600-800 A° with a dense nucleoid (Fig. 49, 52) were also found.

The granulated epithelioid cells presented also the presence of microspheres mostly near of some granules as described in the precedent section of this chapter. Similar vesicles with dense nucleoid as above described were also found.

Other findings, as seen in Figs. 51, 52, showed that the afferent arteriole and the granular epithelioid cells had a rich nerve supply. The specific details concerning the innervation of juxtaglomerular cells will be described in chapter V.

c) DISCUSSION.

As shown by the electron micrographs presented , the granular cells are derived from smooth cells of the afferent arteriole. These findings give evidence to the suggestion of various authors (1, 12, 56, 66) about the smooth muscle origin of the JG cells.

PLATE XVIII

Fig. 49. Enlarged field of TG_2 from fig. 46. Golgi zone (Go). Endoplasmic reticulum (ER). Mitochondria (M). Lysosome-like forms (Ly). Granules (G). Myofilaments (My). Micropinocytic vesicles (Large arrow). Microvesicles with dense nucleoids (Small arrows). x 32 500.

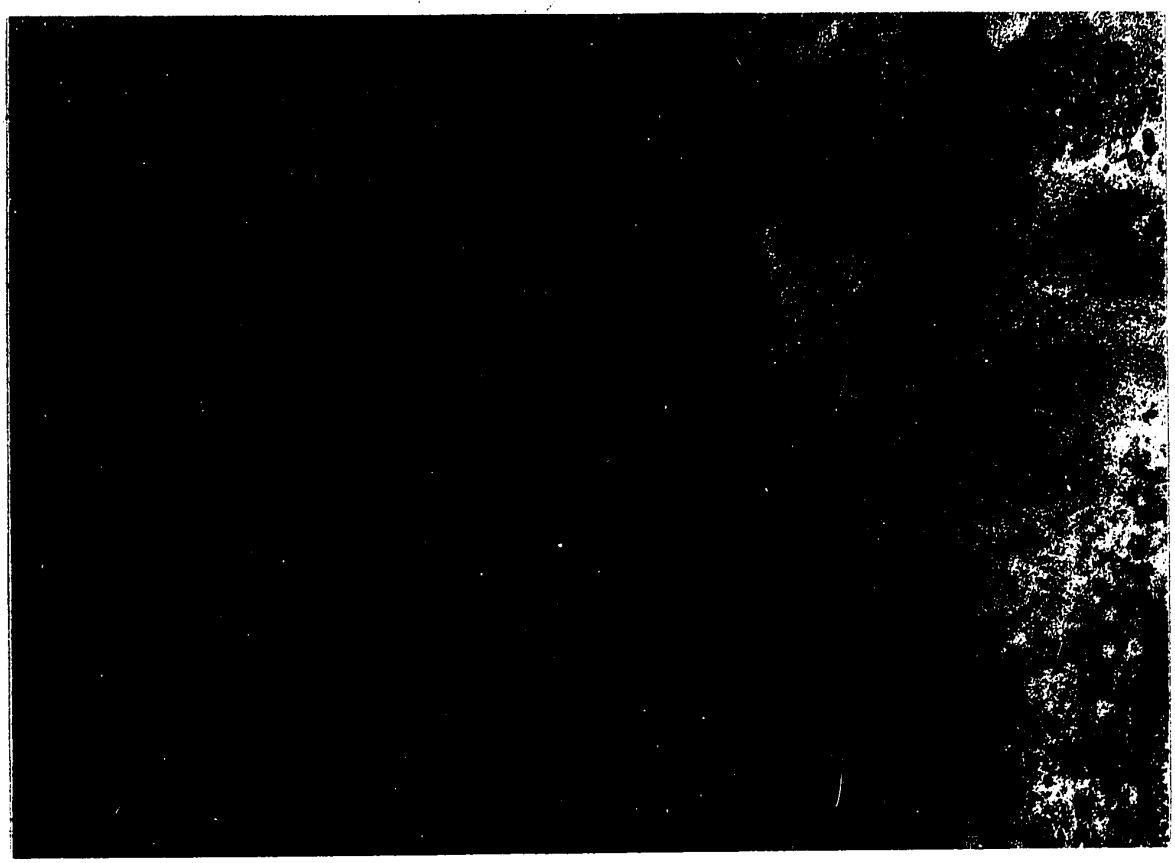
FIG. 50. Enlarged field of TG_3 from fig. 46. The endoplasmic reticulum (ER) is very well developed, several granules (G) are present, but still there are many myofilaments (My) with dense bodies (Large arrows). Microvesicles with dense nucleoids are in the center of the field. x 17 500.

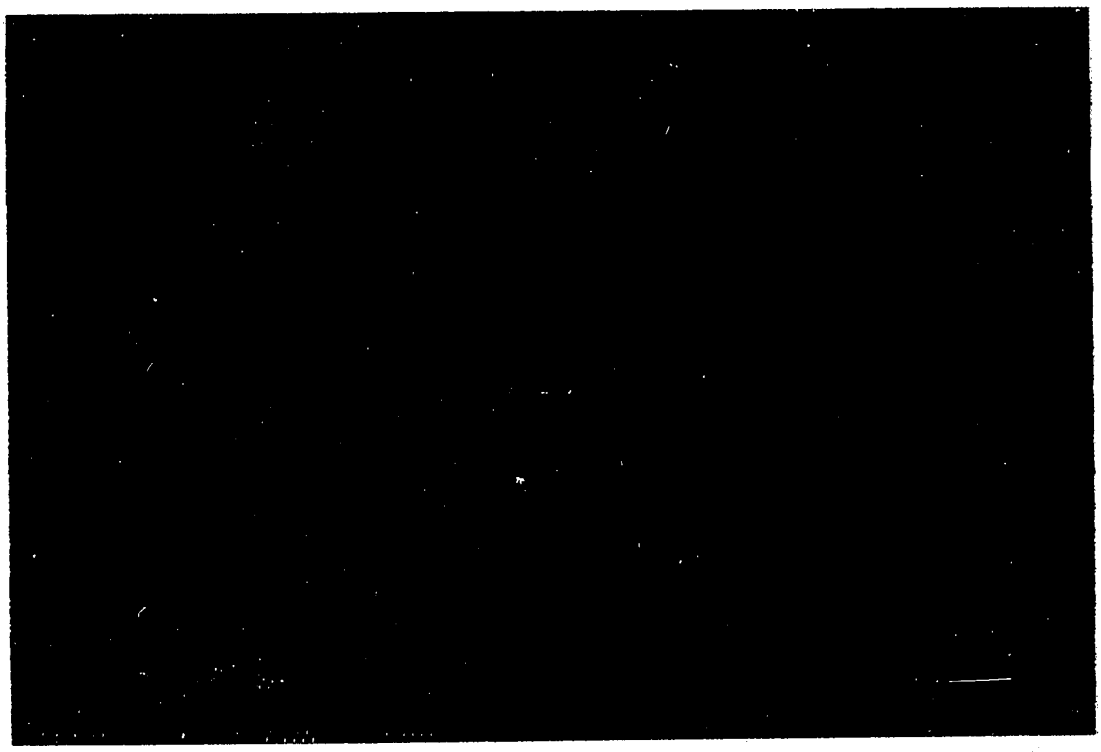
PLATE XIX

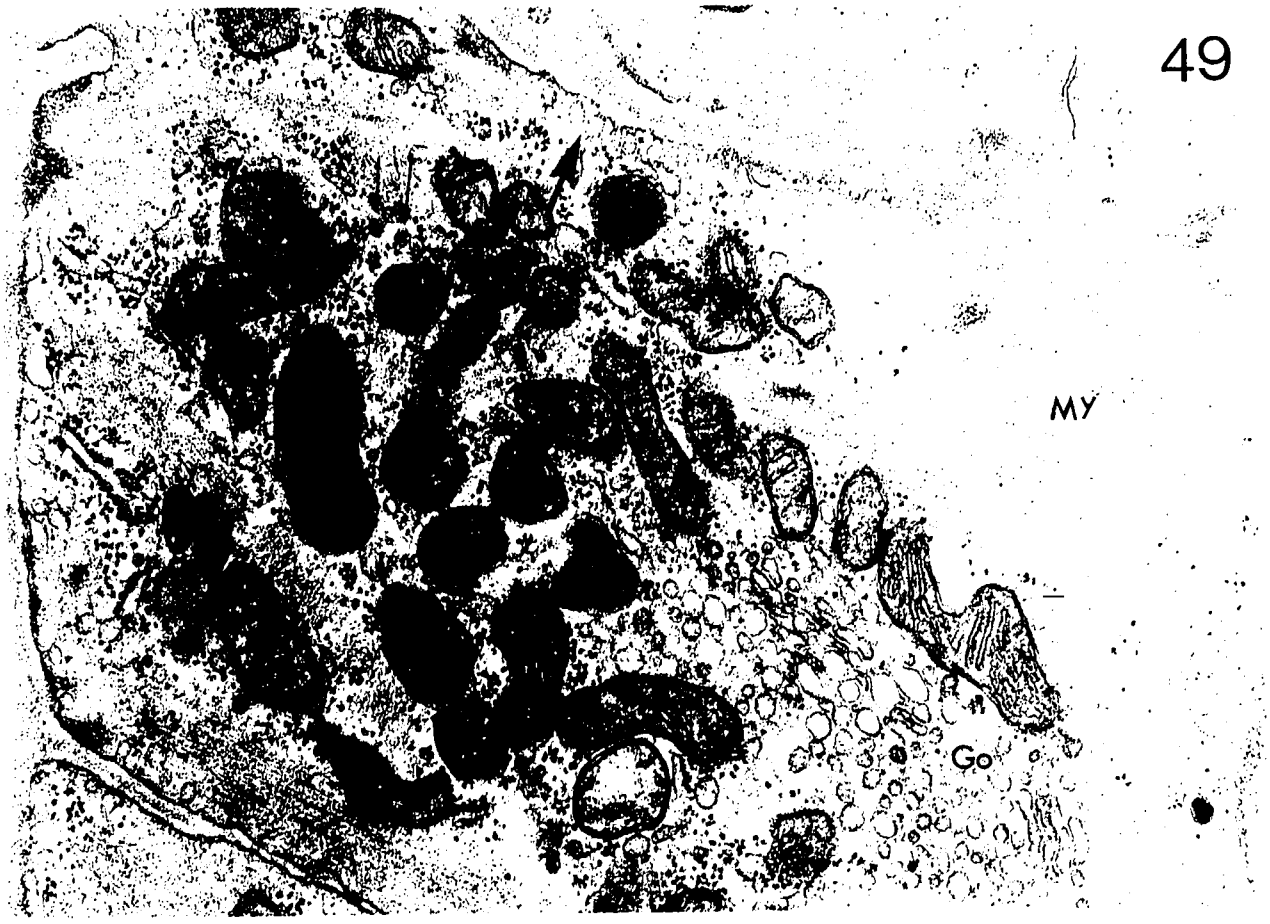
FIG. 51. Enlarged field of TG_4 from fig. 46, showing granules in the neighbourhood of the Golgi apparatus (Arrows). Myofilaments (My). Nucleus (N). On the right, a cell presenting an apparent confluence of various granules. Nerve terminal areas (NA). Endothelium (Ed). x 5 400.

FIG. 52. Enlarged zone from fig. 46, showing a granular cell. Nucleus (N). Granules (G). Golgi zone (Large arrow). Myofilament at the cell periphery. Vesicles with dense nucleoid (Small arrow). Nerve terminal areas (NA). x 5 400.

PLATE XVIII







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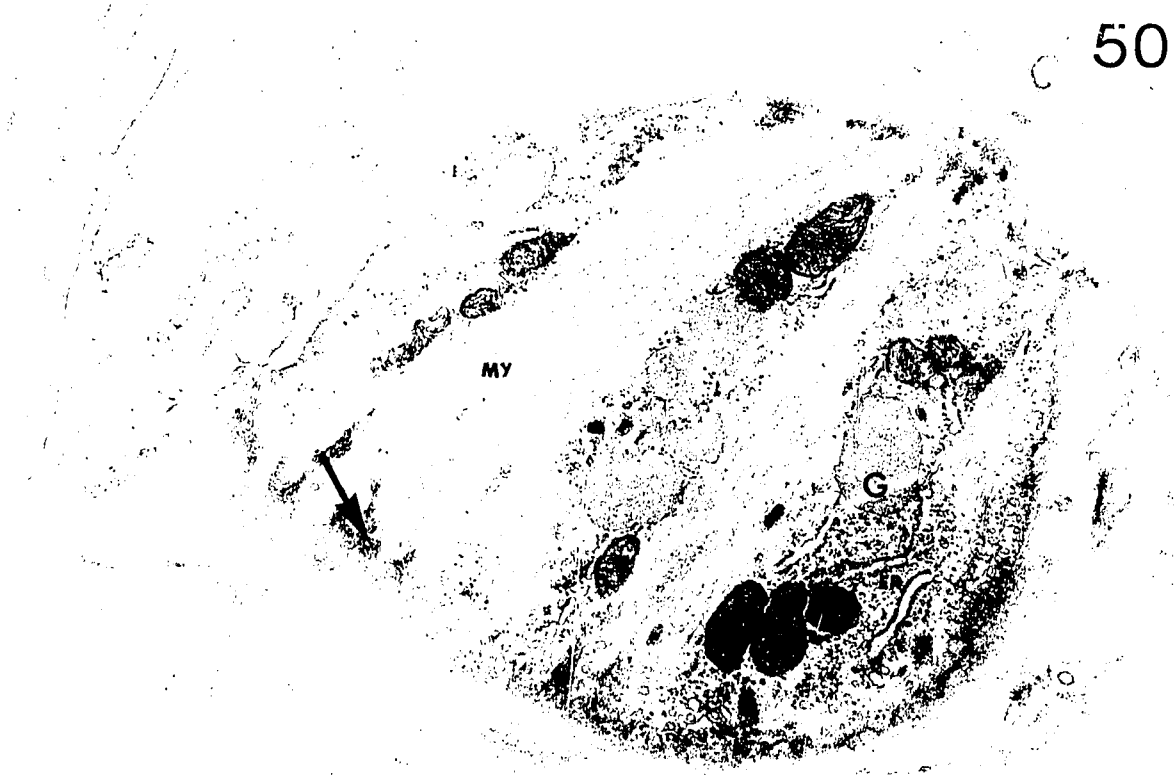

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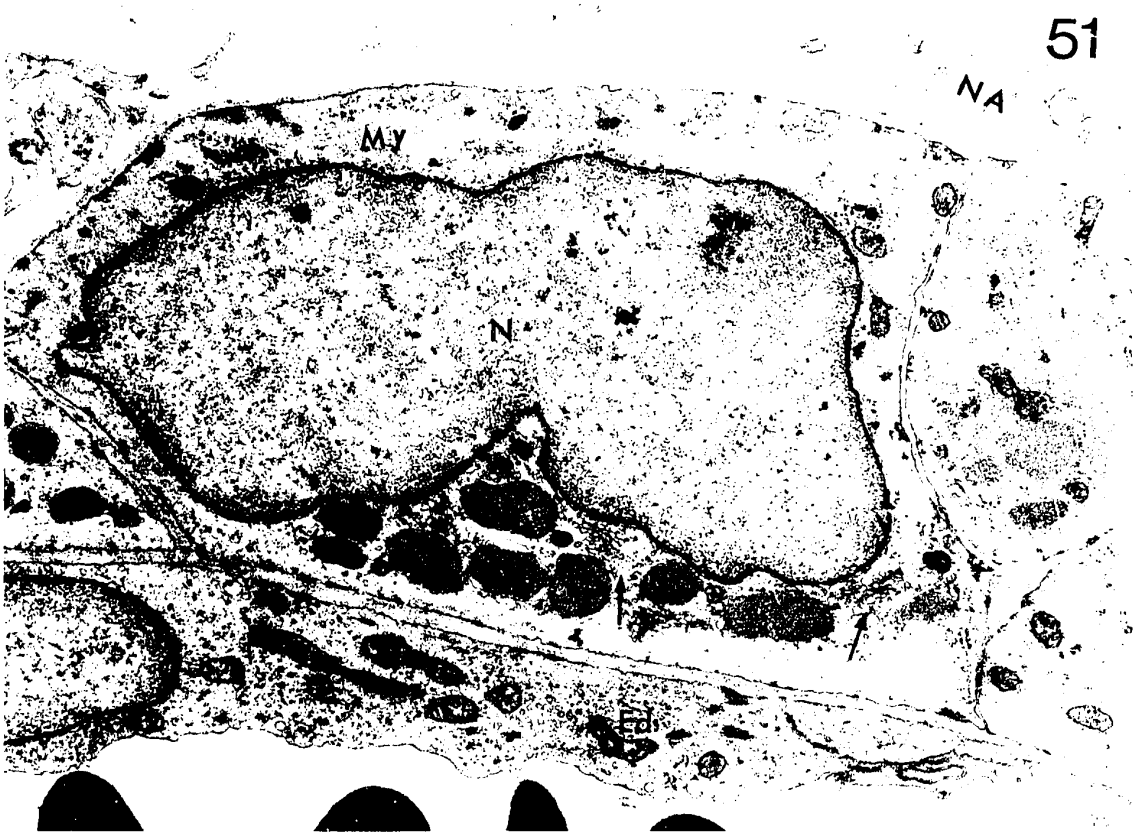
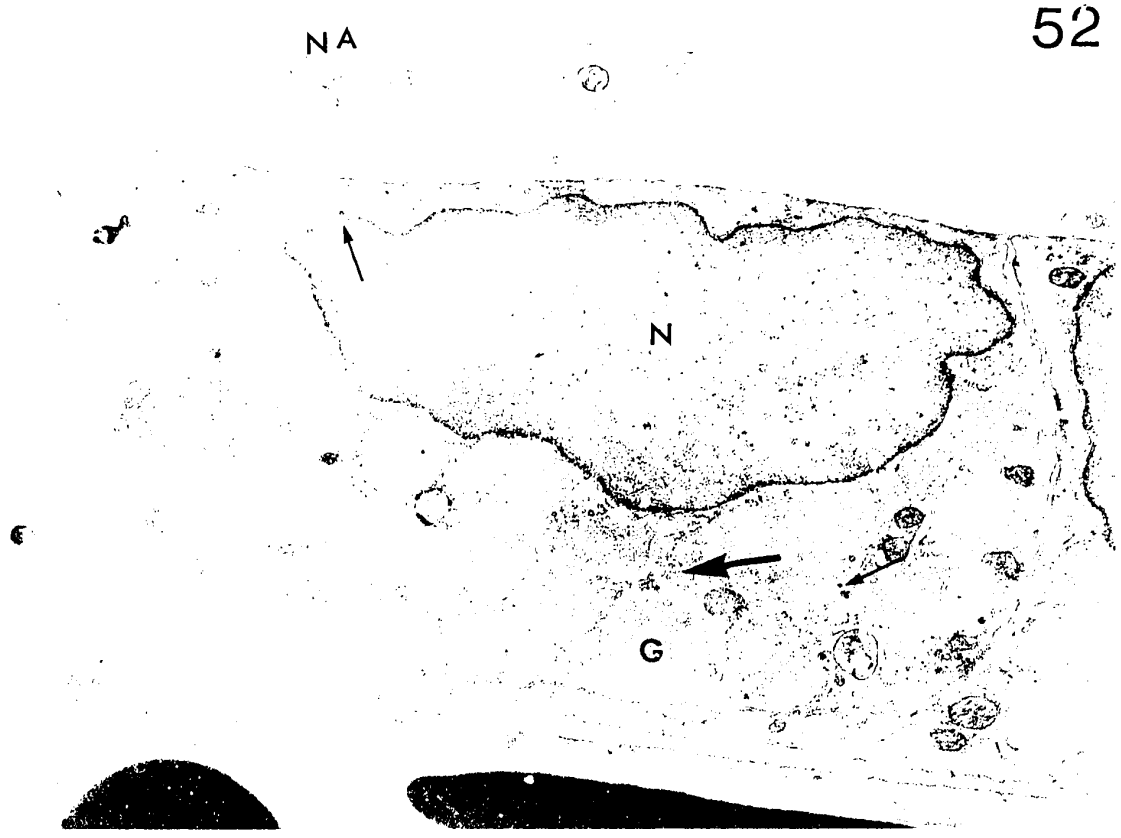
PLATE XIX

PLATE XIX





From our findings, we suggest that the transformation of the smooth muscle cells of the afferent arteriole into granular epithelioid cells appeared to undergo, at least from the morphological point of view, various stages: a) The nuclei and the nucleoli of the smooth muscle cells become enlarged, thus adopting an epithelioid aspect (Fig. 46). These changes are followed by an increase in robosomes, mitochondria and Golgi vesticles and afterwards some granules appear near the Golgi zone (Figs.47, 49-51). b) In the following stage, the endoplasmic reticulum becomes much more developed. The number of granules is increased showing variations in density, form and size, apparently the larger ones are due to the confluence of the smaller ones (Fig. 51). The Golgi apparatus is prominent, the amount of myofilaments appears to be greatly diminished. c) In the next sequence the cell is filled almost completely by the granules and the bundles of myofilaments remain moslty at the periphery of the cell near the cell membrane.

Recently, Lee et al (68) and Fisher et al (70) have demonstrated the presence of acid phosphatase in granules of the JG-cells, suggesting that the JG granules could be identified as lysosomes. Fisher et al (70) suggested that granules of JG-cells might contain other hydrolases that have been found in lysosomes and possibly another enzyme, renin. However, we must consider de Duve's suggestion (589), that, possibly in some specialized tissues, one or more of the enzymes usually found in lysosomes may be associated partly or totally with a special kind of particles unrelated to lysosomes.

Several lines of evidence have shown that the JG cells contain renin (see p. 14), and we have demonstrated previously a correlation between the

JG cells granularity and plasma renin activity. At the present time, it is impossible to say whether or not, the possible hydrolases of the granular content could be in some way related to the release of renin or some biologically active molecules. Recently, the lysosomes have been implicated in the release of thyroid hormones by the demonstration that they could take up the iodinated proteins under the influence of (590) Thyroid Stimulating Hormone and that the lysosomal cathepsin is able to hydrolyze thyroglobulin. Therefore, a similar possibility is suggested in the granules of the JG cells, that is to say, through a specific stimulus the hydrolases contained in these granules, will break the granule membrane and release its contents.

The increased JGI after 72 hours of $HgCl_2$ injection might well be considered as an increase in renin secretion. These findings also suggest that erythropoietin (Ep) is not related to JG cells since Reissmann et al (172) demonstrated in similar experimental conditions that mercuric chloride abolished the ability of the animals to produce Ep as did bilateral nephrectomy. A possible physiopathological role of the JG complex is suggested in the rat with mercury-induced acute renal failure in the micropuncture studies of Flaningan and Oken (591), and Oken et al (592) who found that the anuria or oliguria was associated with a greatly decreased flow rate of the proximal tubular fluid. This was not due to either increased reabsorption of glomerular filtrate or obstruction of the tubules but to an extreme reduction in glomerular filtration. These authors attributed their findings as to an aberration in glomerular afferent-efferent balance. Our observations strongly suggest that these findings might well be related to the renin-angiotensin system.

CHAPTER V

THE INNERVATION OF THE JUXTAGLOMERULAR CELLS IN VARIOUS EXPERIMENTAL STATES. Electron microscopic studies.

The main purpose of this study is to describe in detail the innervation of the granular epithelial cells from our experimental material and discuss the possible synaptic nature and function of these nerve terminal areas.

A) MATERIAL AND METHODS.

The animals and techniques employed were the same as in the sections A and B of the preceding chapter. However, several serial sections were carried out in order to demonstrate close neurovascular relationship.

B) RESULTS.

Our observations were made in control animals (Fig. 53), sodium-restricted animals for 30 days (Fig. 54) and mercury bichloride-treated animals (Figs. 56-62). All nerve fibers observed in this study were non-myelinated.

"Axons" : number.

The loose adventitial connective tissue contains various bundles of axons, accompanied by Schwann cells which usually cover the outer aspect of the axons and sometimes also lie in between them (Figs. 53, 54). The number of bundles around an arteriole is quite variable, and depends on the number of serial sections. However, in mercury bichloride-treated rats, the number of bundles running on the surface of the vessels are

PLATE XX

FIG. 53. Kidney from a normal rat. Light vesiculated nerve process (VP), associated with a granular cell process of an afferent arteriole. The vesiculated nerve process has no Schwann cell (Sc) covering over part of its surface giving directly towards the granular cell but the upper portion of the micrograph is seen to be more deeply incorporated in the Schwann-axon bundle. The axoplasm contains several mitochondria. Small (see text) and large vesicles with dark granules (V_1-V_3). Juxtaglomerular cell granules (G). Note also a small vesicle(Arrow), between the axolemma and the basement membrane of the arteriole. x 17 500.

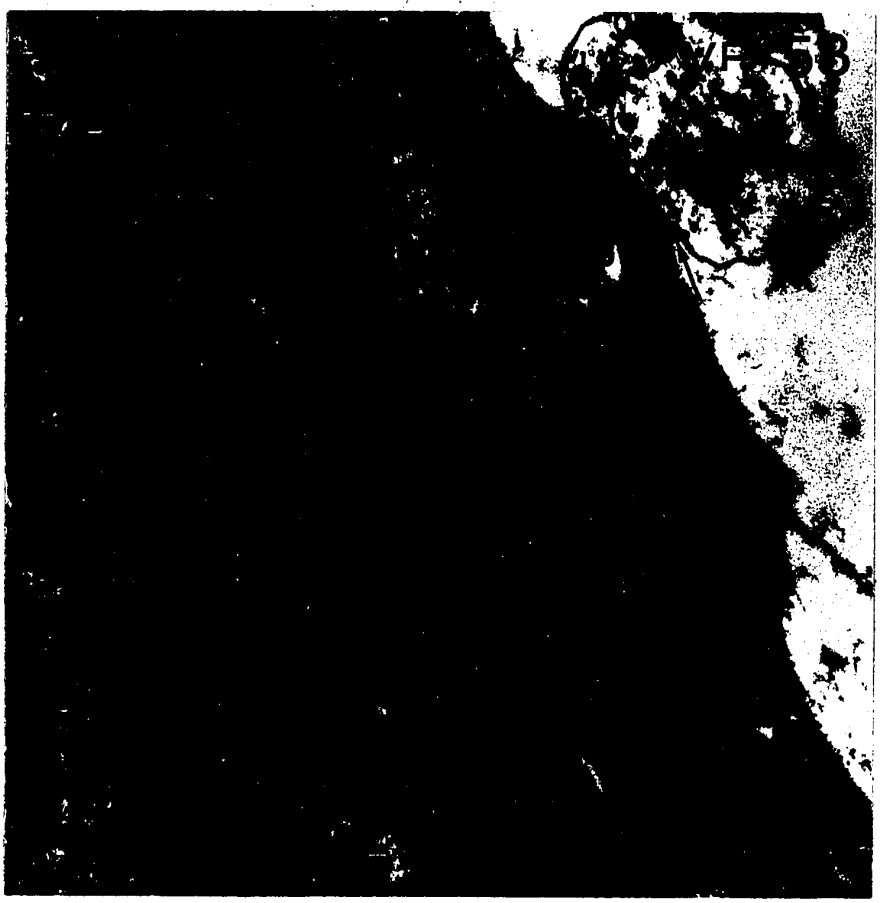
FIG. 54. Kidney of a rat receiving a low salt diet for 1 month. Light vesiculated processes (VP) at the left associated with a JG cell process, and at the right with a granular cell. Schwann cell (Sc). x 17 500.

PLATE XXI

FIG. 55. Higher magnification of fig. 54, showing large vesicles (V_1) and small (V_2). Mitochondria (M). The large arrow points to a possible communication between a small vesicle and the axolemma. Note a small vesicle in the interstitial space (Small arrow). x 50 000.

FIG. 56. Kidney of a rat treated with $HgCl_2$. Various vesiculated processes (Arrows) associated with granular cells. Endothelium (Ed). x 7 500.

PLATE XX



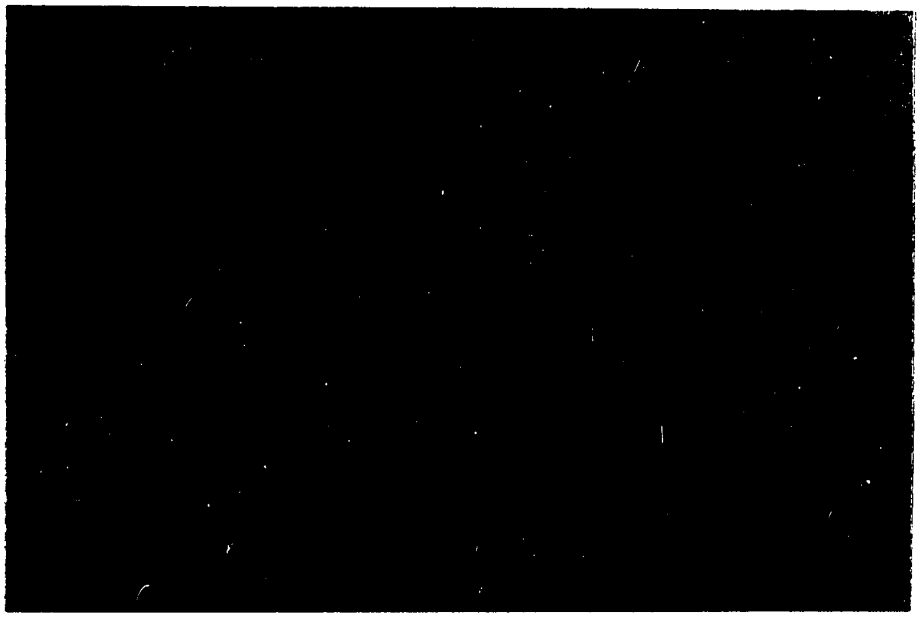


PLATE XV

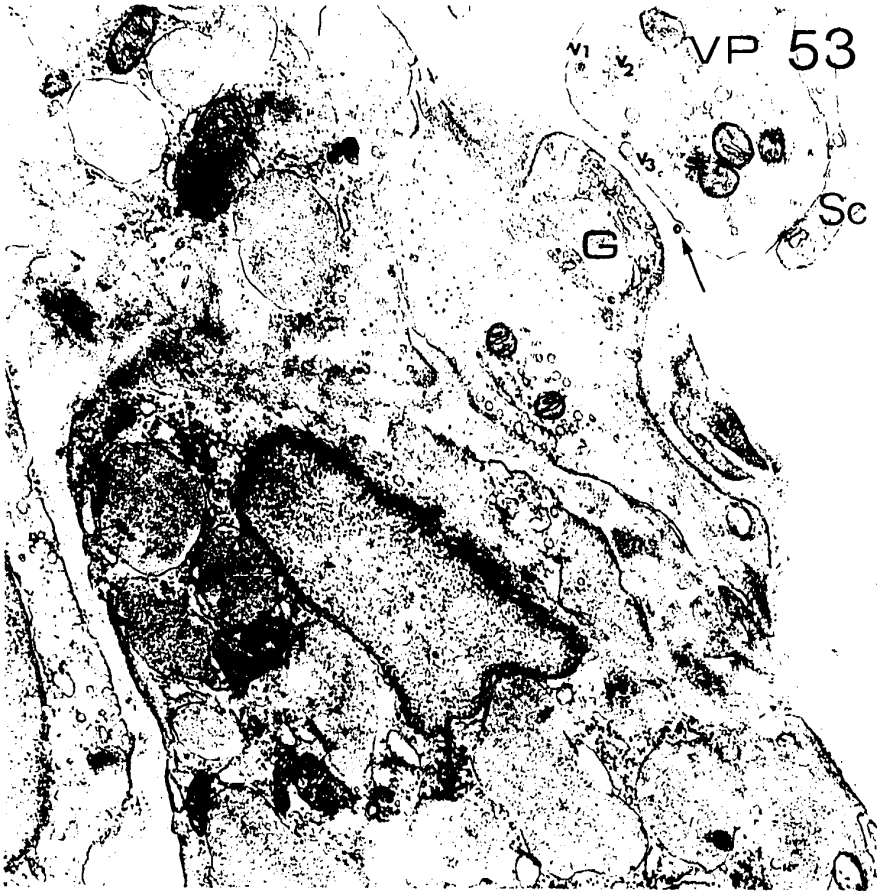


PLATE XXI

PLATE XXI

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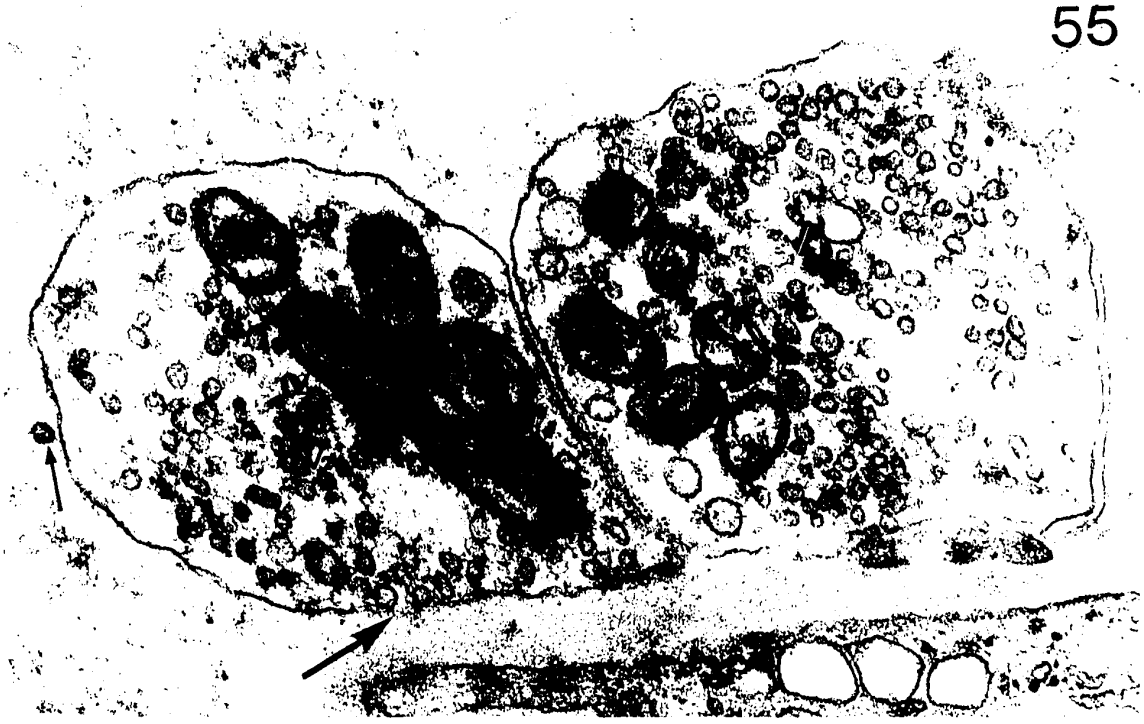

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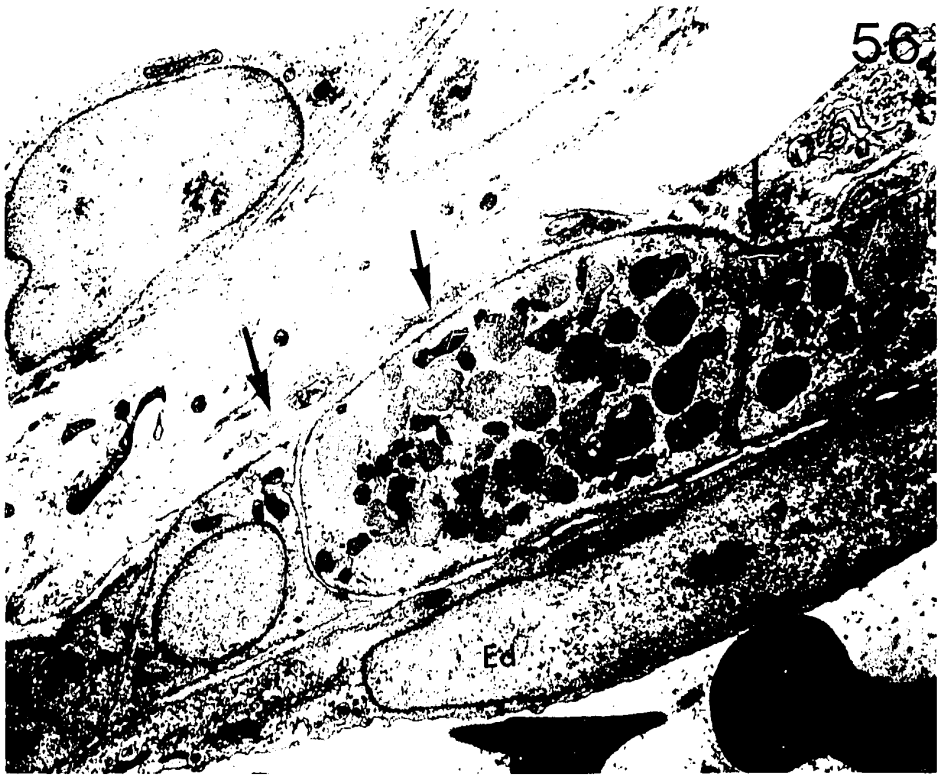


found to be greater than in control and sodium-restricted animals. The axons dilate as they approach the arteriolar wall and form vesiculated sacs or processes (Fig. 54) considered as terminal or near-terminal axons areas.

"Axons relationship to other axons".

Axons dilatations were usually incompletely covered by Schwann cell ; they were often separated from each other by portions of Schwann cells but in many instances a close contact between axons were seen (150-300 A° gap between midpoint of plasma membranes). The distance between the dark layers of the axon plasma membrane and the Schwann cell plasma membrane is relatively constant but occasional expansions are present. Various axons were seen associated with a single Schwann cell (Fig. 58). Collagen fibres were often conspicuous in the area adjacent to the axon-Schwann cell complex.

"Axon relationship to juxtaglomerular cells".

The axon-Schwann cell complex, in which a Schwann cell is associated with numerous axons was found adjacent to the juxtaglomerular granulated cells or to arteriolar smooth muscle cells (Figs. 56-62).

Axons dilatations lie 1000 - 2.000 A° from the outer layer of juxtaglomerular granulated cells, but some examples of closer contact were seen, with gaps of only about 700 A°. This degree of contact could be seen to be maintained over distances of 3 μ or more in some cases (Fig. 59). No examples of contact closer than 700 A° were observed. The space between the axon and the granular epithelioid cells or the smooth muscle cells in such places was filled with a basement membrane-like material. The axon was never seen penetrating the cytoplasm of a smooth muscle cell. However,

PLATE XXII

FIG. 57. Higher magnification from fig. 56. Vesicular processes (VP) showing small and large vesicles. Note communication or disrupted zones between small vesicles and the axolemma (Arrows). Note also a micropinocytic vesicle in the granular cells in front of the disrupted axolemmal zones. Mitochondria (M). JG cell granules (G). x 32 500.

FIG. 58. Higher magnification of the right side of fig. 56. Various vesicular nerve processes (VP). Note in the intercellular space of two granular cells a vesicular nerve process, showing small and large granular vesicles (Arrow). JG cell granules (G). x 32 500.

PLATE XXIII

FIG 59. Kidney of a rat treated with $HgCl_2$. Light (VP) and dark (DVP) nerve processes associated with JG cells of an afferent arteriole. Collagen fibers (Cf). x 7 500.

FIG. 60. Higher magnification of a serial section from the right side of fig. 59. Light vesiculated processes, showing large and small vesicles (See text). Mitochondria (M). Schwann cell (Sc). Granular cell with evident granule confluence (Arrow). x 32 500.

PLATE XXII

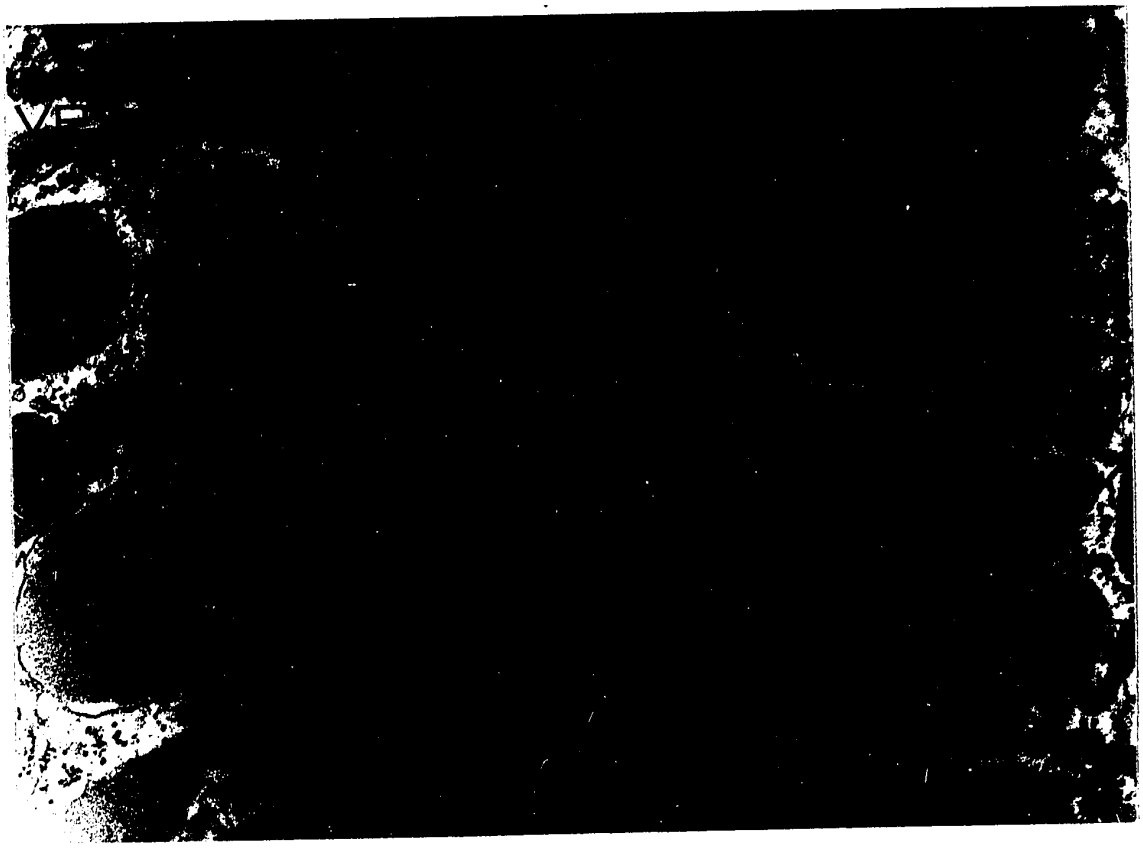
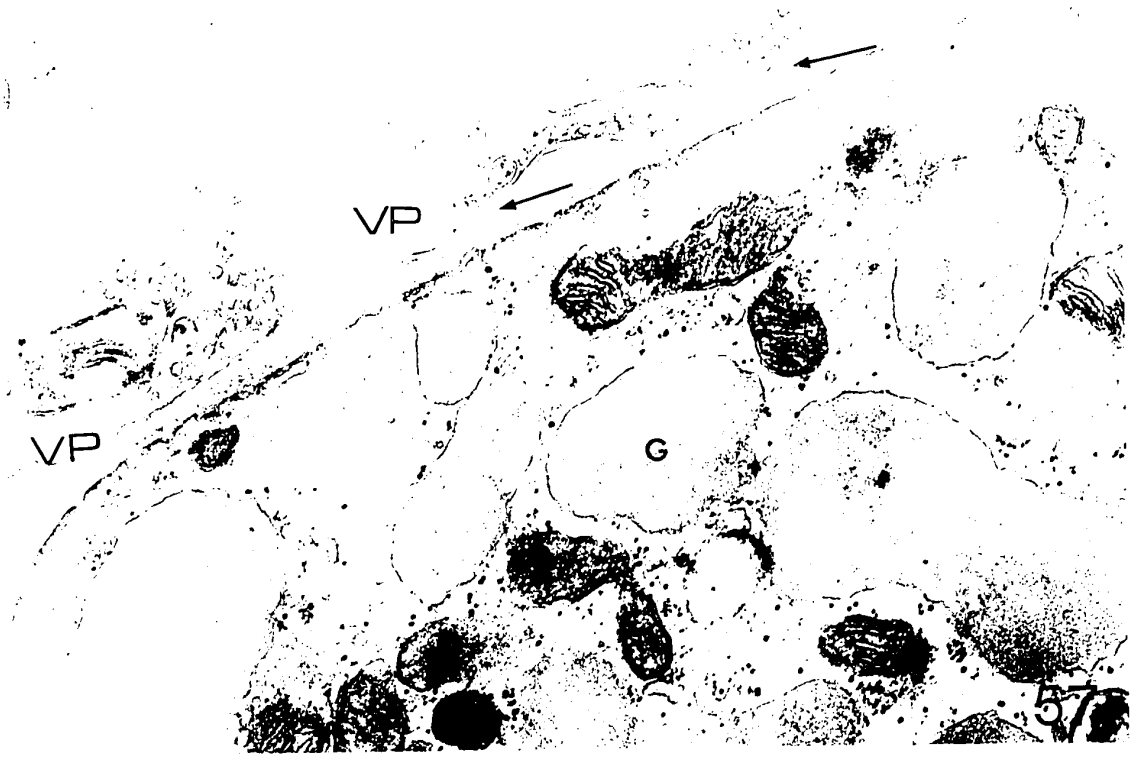


PLATE XXII



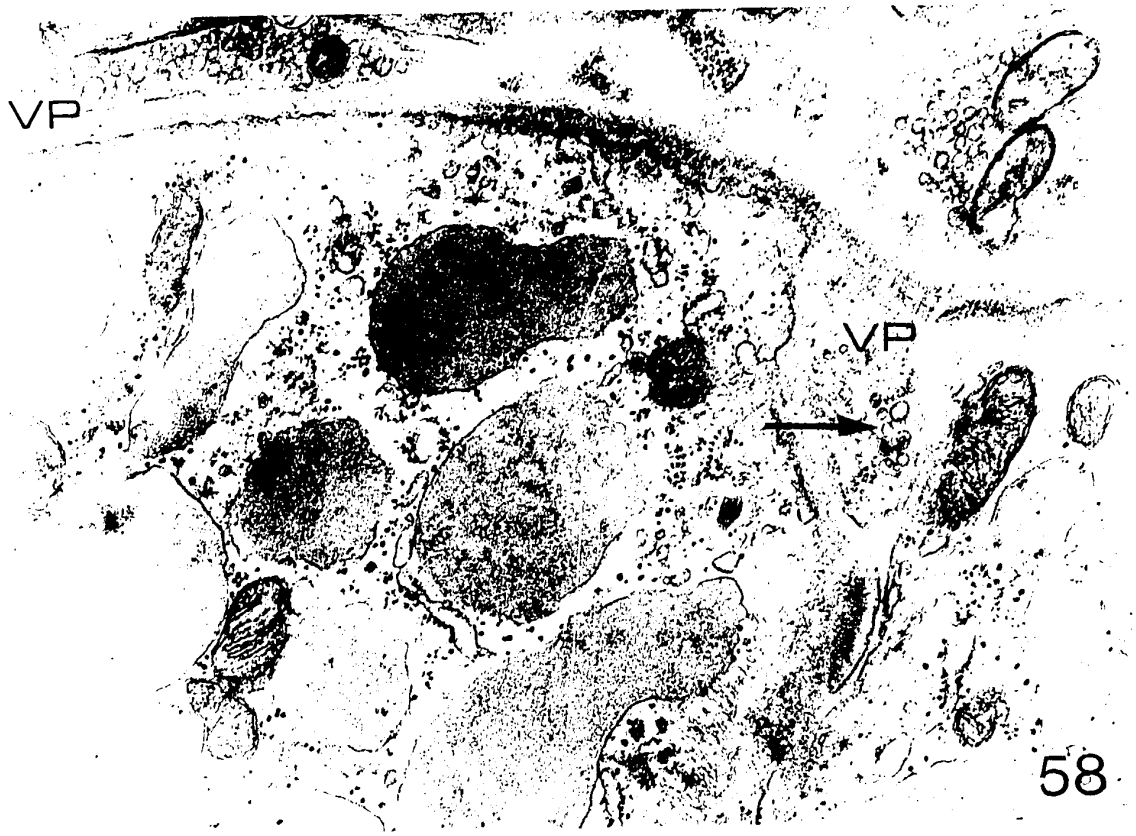


PLATE XXIII

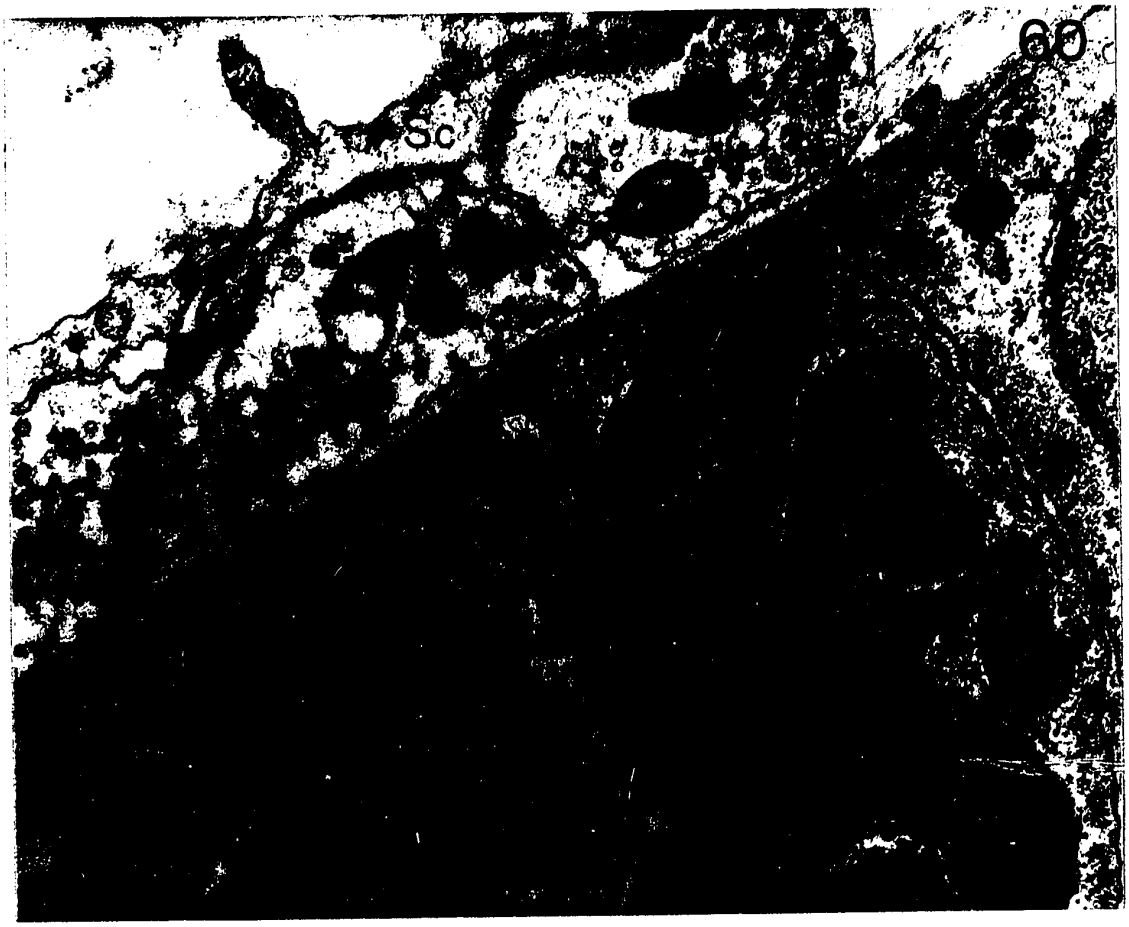
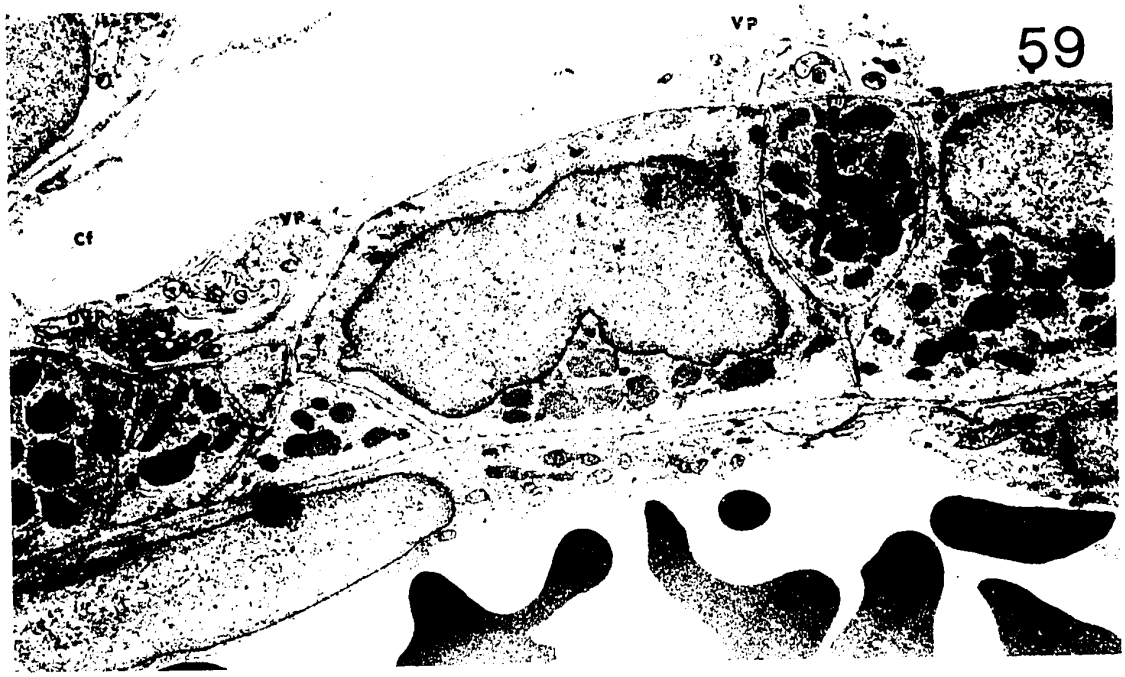
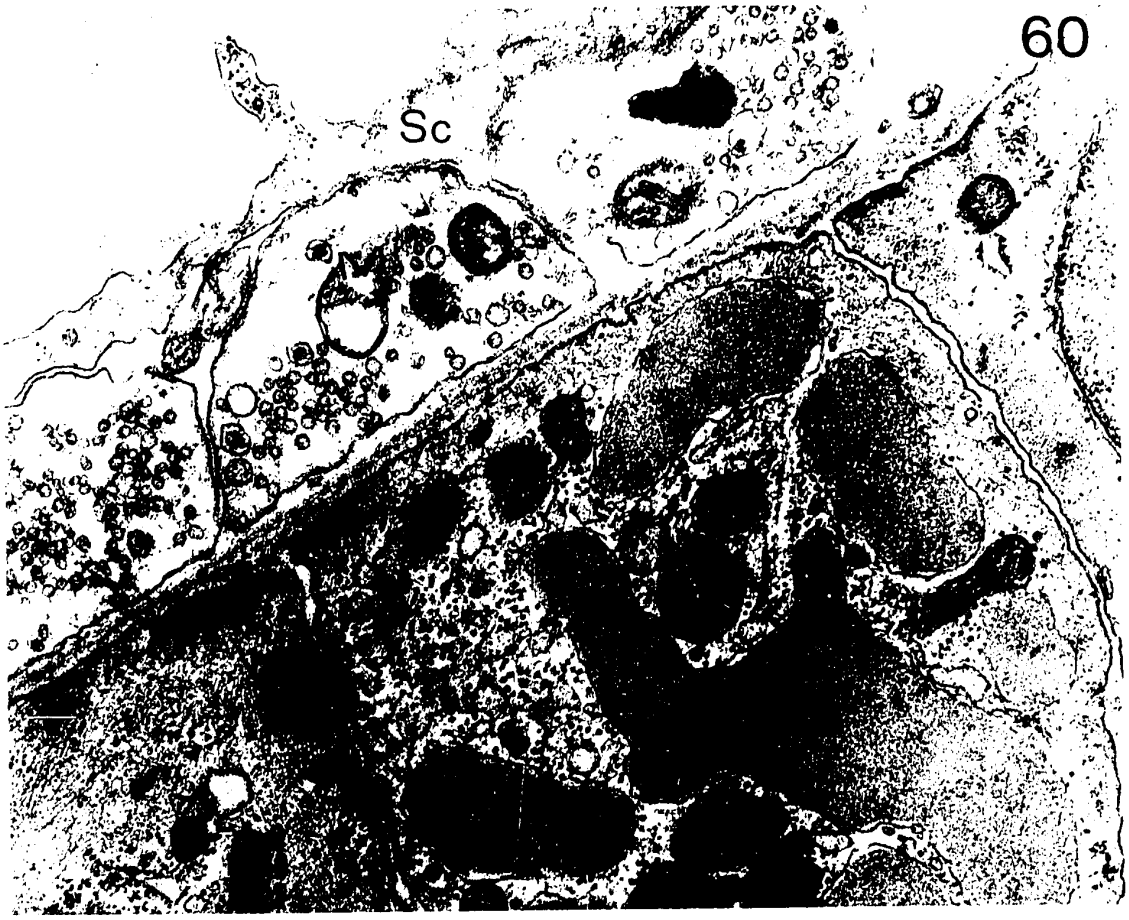


PLATE XXIII





angular projections in between two juxtaglomerular granulated cells could be observed (Fig. 48). From figures 56 and 59 it appears that individual axons may have vesiculated processes or possible nerve terminal areas in relation to more than a granular cell or smooth muscle cells, and it is also apparent that individual granular cells may be closely related to the terminal areas of more than one axon.

"Axon contents".

The axons contained mitochondria, neurofilaments and a variety of vesicles and granules.

The mitochondria are small and often elongated. They are most common on the part of the axons where granulated vesicles were present.

The neurofilaments appear in some sections to be packed at the entrance of the vesiculated sac (Fig. 61). In some axon sections, neurofilaments and vesicles appear to be partly segregated, the vesicles lying in the inner half of the axon nearest the vessels, and the neurofilaments lying in the outer half of the axon (Fig. 61, 62).

The vesicles found in the dilated axons (or sacs or vesiculated processes) are of various types. a) large vesicles, up 800 A° in diameter (TypeV/I) containing, in many cases, a moderately opaque material and usually also a small granule of varying density and of frequently irregular shape (Fig. 53). b)Smaller vesicles 500-600 A° in diameter containing a moderately dense material (TypeV/2) (Fig. 53), and c) other vesicles of similar size (500-600 A°) and density but containing a tiny granule or core (Type V/3). The type of vesicles most frequently encountered in our experimental material were the type V2. The least frequently seen were those of Type V3.

In the mercury bichloride-treated animals, some vesiculated processes vary greatly in electron density. Most of them appear relatively light, (Figs. 60 and 61), but few of them were relatively dark (Fig. 62). This difference in electron density seems to be related not only to a greater concentration of organelles (mostly vesicles of type VI2) but also to a darker cytoplasm between them. The light processes contain similar elements separated by a light cytoplasm. They appear to contain relatively more vesicles of type VI. Dark and light vesiculated processes may be seen as part of the same axon-Schwann cell complex. In normal and sodium-restricted rats, no dark processes were found.

The total number of microvesicles when comparing one experimental animal to another is difficult to evaluate, since there are wide variations not only from one animal to another but also between comparable axons within the same animal.

The microvesicles in saccular processes or terminal axons seems to be more numerous in the zones being in contact or direct comminication with the plasma membrane on the uncovered aspect, facing towards the granular cells, than on the aspect which has a Schwann cell covering. A "streaming" or crowding of microvesicles towards the uncovered surface is often seen as a possible characteristic feature of terminal axons. Membrane communications (Figs. 57 and 58) between axolemma and small vesicles were quite often found in animals treated with mercury bichloride. No evidence of such membrane communications were observed in control or sodium-restricted animals. However, in the latter, the basement membrane may be occasionally attenuated, thus giving the impression of possible communications (Fig. 55).

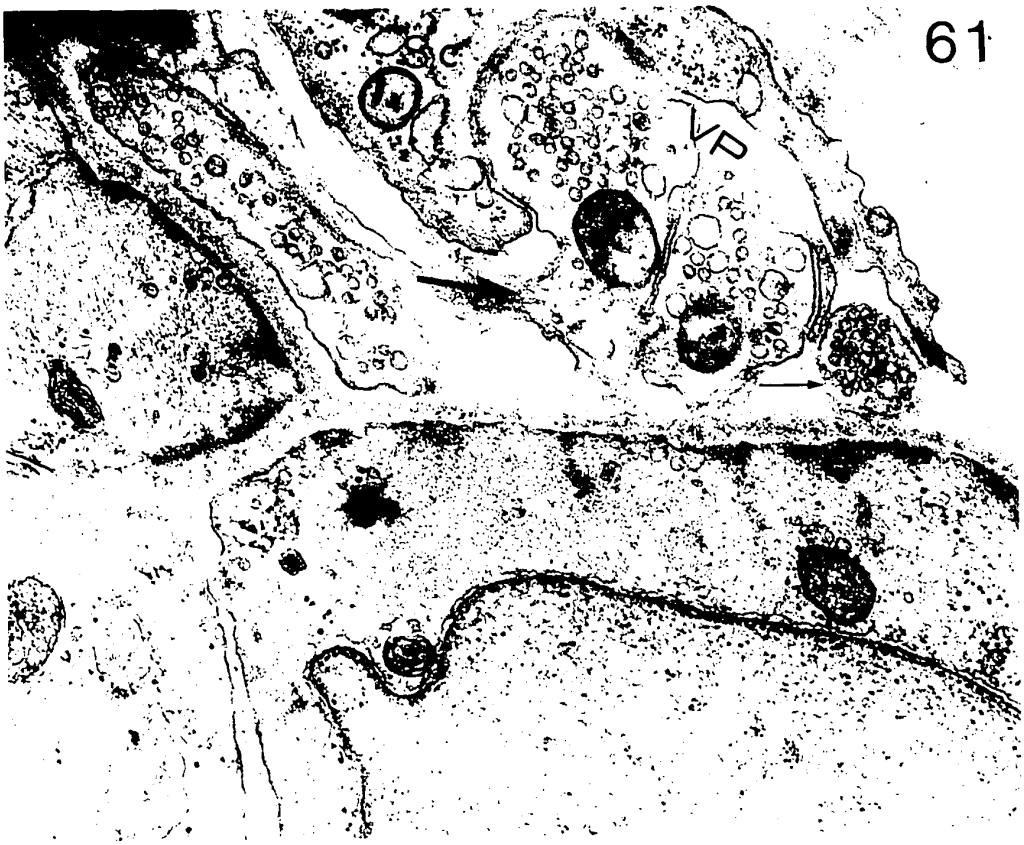
PLATE XXIV

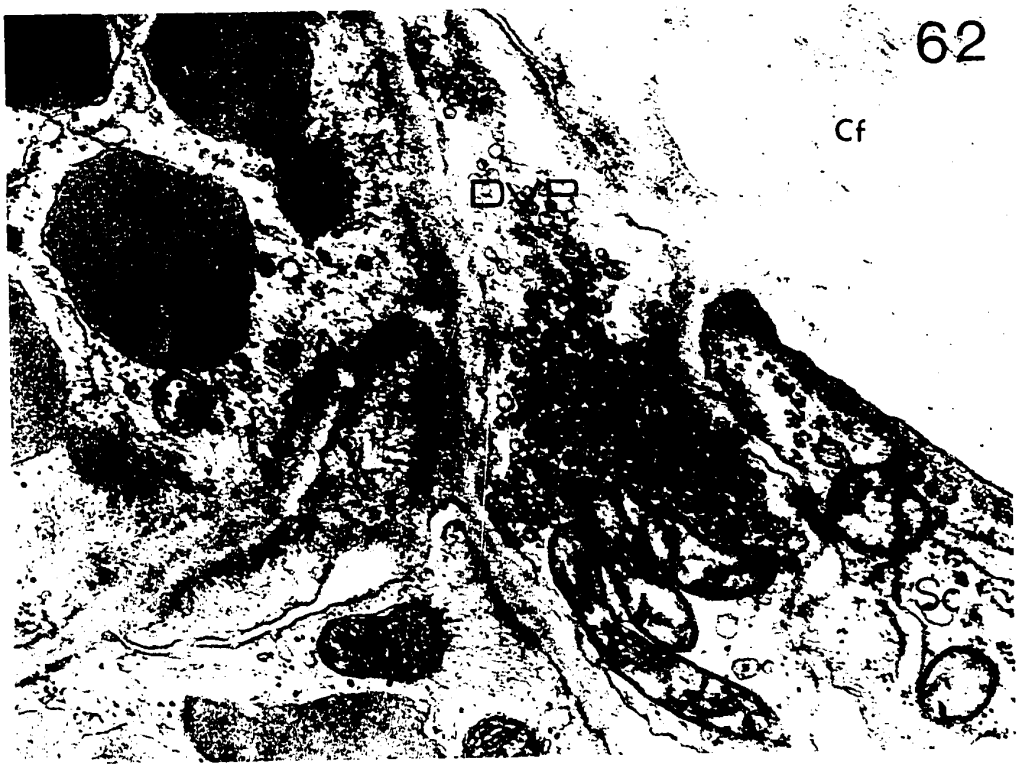
FIG. 61. High magnification from fig. 59. Light vesiculated nerve processes (VP). Neurofilaments (Large arrow). Schwann cell (Sc). Dark vesiculated process (Small arrow). x 32 500.

FIg. 62. High magnification from fig. 59. Dark vesiculated process (DVP). Note that both this dark vesiculated process and the light of fig. 61.form part of the same axon-Schwann complex. Schwann cell (Sc). Mitochondria (M). Juxtaglomerular cell granules (G). Collagen fibers (Cf). x 32 500.

PLATE XXIV

PLATE XXIV





It is difficult to be more precise, since they were often observed in electronmicfographs, within areas of increased osmophilic extending across the axolemma. Rarely, a single vesicle of the same characteristics as the type VI was observed in the space interval between the axolemma and the JG cell membrane (Fig. 53).

c) DISCUSSION.

Direct evidence showing the fine neuro-granular epithelioid relationship in the juxtaglomerular apparatus of the rat has been lacking from the few electron microscopic studies already published by other authors (364, 365).

In the monkey, Barajas (364) has shown light and dark vesicular processes separated from the granular cells by an interval which varied in width from 1000 to 2000 A°. However, this author did not show in the rat vesiculated axons in contact with the granular epithelioid cells, and his electron micrographs showed only a light vesiculated nerve process near a smooth muscle cell, concluding that few nerve sacs associated with the juxtaglomerular apparatus are present in the rat.

Our findings in rats have shown a relatively rich nerve supply of the juxtaglomerular granulated cells. This nerve supply was more marked in animals treated with mercury bichloride, in which dark vesiculated processes similar to those described by Barajas (364) in the monkey were observed. However, their possible relationship with kidney nerve fibres exhibiting different affinities for silver stains as described in light microscopic studies (282, 288, 360) remains speculative.

1) Possible synaptic nature of the vesicular nerve processes in the arteriolar wall.

Electron microscopic studies of synapses in the central nerves system have revealed a number of characteristics. Presynaptic axonal dilatations are seen with mitochondria and large number of synpatic vesicles (523, 524, 525, 526). Similar vesicles are described in the receptor synapses of the retina (527). A presynaptic thickening of the axoplasmic membrane is separated from the postsynaptic membrane by an interval synaptic cleft measuring approximately 200 A°. Gray (523) found neurotubules in certain axonal terminals of the visual area of the cortex. Robertson et al (528) also found neurotubules in the presynaptic axoplasm of the club ending of Mauthner cells of the goldfish. In peripheral sympathetic ganglia (529, 530, 531, 532, 533, 534, 535) a similar presynaptic dilatation of the axoplasm containing clusters of synaptic vesicles has been noted. They also contain mitochondria, large vesicles with a large spheroidal droplet of finely granular texture and variable density (533, 534), and small vesicles containing irregular dense granule or core (533).

In the sympathetic ganglia of the bullfrog, the plasma membrane in the presynaptic axoplasm presents zones of thickening in some areas of membrane and membrane contact (534), in the postsynaptic axoplasm adjacent to this area of thickening, Taxi (534) has found a sub-synaptic plate measuring 0.5 μ in length and 500 A° in thickness. In the sympathetic ganglion of the cat, Eflwin (531, 532) reported dense osmophilic material of varying thickness associated with cytoplasmic surface of the postsynaptic membrane.

Based on a single observation Thaemert (536, 537) stated that the intimate neurovascular relationships on the vascular walls "seem to be rare", suggesting that membrane-to-membrane intervals wider than 770 A° are not functional neuromuscular junctions. However, it should also be recalled that neuromuscular junctions of the striated skeletal muscle are not a membrane-to-membrane contact. There is a primary and secondary synaptic cleft interposed between the plasma membrane of the nerve ending and that of the muscle cell. The cleft is occupied by a basement-like material, referred to as an "amorphous surface material" by Zachs and Blumberg (538).

Fawcett and Selby (541) have shown micrographs of the turtle atrium depicting intervals of about 1500 A° between pre and postsynaptic components at presumptive nerve terminals : they do not specify whether the terminals are adrenergic or cholinergic in nature. Richardson (539) and Jabonero (540) have suggested that axons passing close to muscle fibers may not possess definite endings but release transmitter substances at intervals along their length.

The innervation of the arterioles in general, as demonstrated by electron microscopy, has received the attention of a number of authors. Some studies of the innervation of the vascular smooth muscle in mammals have been reported by Caesar et al (542) and by Rhodin (543) and Zelander (544). However, the first account of an electron microscopic study of presumptive vasomotor nerves was reported in 1961 by Lever and Esterhuizen (545). Working on pancreatic arterioles, they described vesiculated axons lying in the adventitial coat which in places are not covered by Schwann cell processes and are separated from smooth muscle only by basement mem-

brane at intervals which varied in width from 1.000 to 4.000 A°. Later, Lever et al (546) reported in pancreatic arterioles a closer neurovascular interval from 830 to 4 000 A°. Similar findings have been reported in heart arterioles (547), and in kidneys arterioles (364). Appenzeller (548) has described the nearest point of nerve processes to muscle on arteriolar walls of rats as 600 A°. In the present investigation intervals of 700 to 2 000 A° have been found.

In spite of the divergences which might be accounted for by differences in embedding procedures and optical variation in electron microscopes between one laboratory and another, strong evidence has been adduced from various and independent sources pointing to the fact that the shorter distance of nerve to muscle on arteriolar walls in mammals is about three times wider than the typical intrasynaptic space found at myoneural junctions in skeletal muscle (550) and at other cholinergic nerve endings. However, Hartroft et al (365) claimed to observe in the frog, membrane-to-membrane contact of a nerve vesiculated process with projections of juxtaglomerular cells.

No zones of thickening of the axoplasma membrane in the contact region with smooth muscle cells have been found in this study or in others.

We have observed, in two instances, however, vesicular processes in-between two granular cells of the afferent glomerular arteriole (Fig. 58), indicating that, at least in a few cases, the ultimate neurovascular relationship might occur in the intercellular space limiting the modified smooth muscle cells. Similar observations have been described by Thaemert (551) in the external muscularis of the intestinal wall of frogs and in the renal arteries of sheep (548). However, the most frequent finding in this study,

which agrees with other investigators, is that the ultimate neurovascular relationship occurs across the adventitia.

Apparently, "the synaptic junction" between muscle and nerve in the vascular walls of mammals seems different from that in the central nervous system and peripheral ganglia.

The question of membrane continuity between microvesicles and axolemma has not been completely settled by the present study. This, while in many instances this continuity could not be demonstrated, in others, it was at least strongly suggested (Fig. 57). A similar observation was also made by Lever et al (546) in the vesiculated axons in relation to arteriolar smooth muscle in the pancreas, suggesting the probability of humoral release into the neurovascular interval.

Another interesting finding of this investigation is the fact that individual arteriolar nerves have an intimate and significant relationship to granular epithelioid cells over relatively extensive terminal areas. At these areas, axons are denuded of Schwann covering and separated from muscle by basement membrane within an interval varying from 700 to 2.000 A^{o}. It seems that (Fig. 59) more than one granulated cell may be served by a single axon and several axons may serve various individual muscle cells (Fig.56). Similar results have also been reported by Richardson (552) in the vas deferens and by Lever et al (546) in arterioles of the pancreas. According to Lever (546) it is inappropriate to refer to nerve endings on arteriolar walls. Instead those parts of axons bearing the intimate relationship with smooth muscles described in this study should be referred to as""nerve terminal areas".

The issue of whether these nerve terminal areas are synaptic in nature

could not be definitely proven by this investigation. Nevertheless, the abundant incidence of microvesicles at the proximity of the surface contact or the appearence of zones of discontinuity in the axolemma, would lend support to the contention that these vesicular processes might be synaptic in nature.

Definite proof about the identity of the nerves described has not been provided in this investigation and it would therefore be unwise to do more than speculate upon the nature of the material contained within the microvesicles at their terminals. Nerve processes of the autonomic nervous system may contain "synaptic" vesicles ranging in number from a few to many. Such vesicles, ranging in size from 300 to 1 000 A^{o} in diameter, are described as agranular or granular, depending on the presence or absence of electron-opaque-cores. These cores may vary in diameter from 200 to 800 A^{o}.

Granular vesicles are prevalent in a) the nerve processes within the smooth muscle tissue of the vas deferens (552, 553, 554), b) arterioles (545-547), c) dilator muscle of the iris (555) within the adrenal medulla (556), d) pineal gland (557), and e) cardiac muscle (558, 559, 560).

Nerve processes in which agranular vesicles are prevalent have been observed in a) smooth muscle tissue of the gastrointestinal tract (533, 531, 535, 554, 561) and b) sphincter pupillae (562, 563). Only agranular vesicles have been reported to be present in the nerve processes of the smooth muscle tissue of the urinary bladder (537), and ciliary muscle (554, 564).

It has been found with the use of fluorescence methods (565, 566, 567, 568, 569) for the histochemical demonstration of catecholamines, that nervous and nerve processes which contain catecholamines are present in various visceral organs. These methods have shown that the more intense the fluorescence,

the greater the amount of catecholamines. Electron microscopic studies, have revealed that those organs whose nerve processes exhibit intense fluorescence are predominantly endowed with granular vesicles.

Nerve processes containing granular vesicles are infrequently seen in the muscularis externae of the gastrointestinal tract. This finding correlates well with the fluorescence study of Norberg (568) who showed that fluorescent fibers were scarce in the muscle layers of the intestine. However, he did detect intense fluorescence in the mesenteric plexus in which nerve processes with granular vesicles are more frequently found. Nilsson (363) using a similar method, described nerve terminals in the small arteries of the kidney including the afferent arteriole, and considered them as adrenergic. These observations fit well with the electron microscopic studies of Barajas (364), Hartroft (365) and ours.

Cholinergic fibers have been identified using the thiocholine method and they have been detected in the anterior segments of the rabbit eye (567). Heavy staining for nerve terminals (or processes) containing acethylcholinesterase was seen in the sphincter pupillae and ciliary muscle. According to electron microscopic studies, these structures contain nerve processes which are predominantly endowed with agranular vesicles (554, 562, 563, 564).

Von Euler and Hillarp (570) and von Euler and Heller (571) have shown that noradrenaline is in a granule-bound form in homogenates of adrenergic (splenic) nerves. They further suggested that each of these granules is covered by a membrane, since rapid release of noradrenaline into free solution occurs when granule suspension were treated with detergents. Following injections of norepinephrine into rats, Potter and Axelrod (602) found that

the endogenous and tritiated norepinephrine in homogenates of heart, vas deferens, submaxillary and pineal gland were primarily localized in particles associated with the microsomal fraction. In an electron microscopic study of rat hearts, Michaelson et al (572) observed that vesicles with diameter of 500 A^{o} were present within the microsomal fraction. A few of the vesicles contained electron-opaque cores. This evidence seems to implicate vesicles in the storage of catecholamines within the tissues of the heart. Wolfe et al (573, 574), with the use of autoradiography and electron microscopy have shown that, after the injection of tritiated norepinephrine into rats, sections of their pineal glands revealed that only nerve processes containing granular vesicles accumulated the injected norepinephrine. This observation prompted these investigators to conclude that norepinephrine resides in the electron-opaque core of the granular vesicles and that the presence of the latter can be used as a criterion for the identification of adrenergic sympathetic axons in electron micrographs.

It appears from the foregoing discussion that it may be possible to distinguish between sympathetic and parasympathetic nerve processes on the basis of their vesicular content. The present investigation of the granular epithelioid cells shows clearly the presence of granular vesicles of various sizes within the terminal axons.

A significant accumulation of microvesicles upon the free surface of these axons nearest to granular epithelioid cells does occur. It seems possible, therefore, that the juxtaglomerular granulated cells are supplied by an adrenergic innervation.

2) Possible role of the nervous system in the juxtaglomerular cells.

Several lines of investigation strongly suggest that the nerves asso-

ciated with the afferent arterioles may have a significant influence on the regulation of the secretory activity of the juxtaglomerular cells and possibly on the regulation of glomerular circulation and systemic blood pressure. Elaut reported in 1934 (491) that hypertension produced in dogs by section of the carotid sinus is accompanied by hyperplasia of the epithelioid cells of the afferent arterioles, Chernigouskii (575) found an increase of renal renin content within one and one half month after section of carotid sinus nerves, and disappearance within three weeks after kidney denervation. Ratner and Eisengardt (576) studied the effect of renal denervation on renal blood flow, glomerular filtration rate and renal renin content of normotensive rabbits and in rabbits with section of carotid sinus nerve. In the second and third week there were no significant changes in renal blood flow and glomerular filtration rate. The renal denervation produced a decrease in renal renin in both normotensive and hypertensive animals. However, the drop of the renin content was more pronounced in the hypertensive animals because of their increased initial level. Introzzi et al (578) have claimed that renal denervation causes depletion of renin-like activity. Tobian (492) has found in rats following a unilateral renal denervation a decrease of the JGI of granularity and of renal renin content. Taquini et al (202) described a decrease in renin release in dogs after renal denervation. Vander (496) demonstrated that direct electrical stimulation of the renal nerves increased renin release. Bunag, McCubbin and Page (579) and Hodge, Lowe and Vane (497) demonstrated increased renin release and plasma angiotensin-like material respectively in dogs during "non hypotensive" hemorrhage and found that this release

could be prevented by ganglionic blockade or local anesthesia of the renal nerves. Vander and Luciano (580) have reported that renal nerves are of importance in the renin response to acute salt depletion produced by chloromerodrin. The innervated kidney secreted large quantities of renin in contrast to that liberated by denervated kidneys. Furthermore, Wathen et al (494) and Bunag, Page and Mc Cubbin (581) have found that acute infusions of catecholamines in dogs produce an increased renin release. Acute degranulation of JG cells has been demonstrated under similar conditions of catecholamine infusion in cats (582) and in rats (495).

Augmentation of the granular cell index in the rat by either short or prolonged dietary salt restriction has been prevented by reserpine or by norepinephrine injections (498).

It appears from the foregoing discussion that the sympathetic nervous system has an important relationship to the juxtaglomerular cells and the secretion of renin.

CHAPTER VI

A) THE JUXTAGLOMERULAR COMPLEX IN "ENDOCRINE KIDNEY" OF RATS WITH OR WITHOUT HYPERTENSION. LIGHT MICROSCOPIC STUDIES.

There still remains considerable doubt as to the nature of the stimulus which causes the morphological changes in the juxtaglomerular cells (stretch receptor theory, or tubular osmolarity theory through the macula densa). One way in which additional information might be provided, would be the use of an experimental model in which no filtrate reaches the macula densa : the "endocrine kidney".

a) MATERIAL AND METHODS.

The experiment was performed in Sprague-Dawley rats of both sexes and divided into 2 groups. Each rat was kept in an individual metabolic cage, on standard laboratory purina, and tap water "ad libitum".

Group I. "Endocrine kidney" (EK) : 30 rats with satisfactorily produced "endocrine kidneys" comprise this group. In 12 rats weighing 140-150 g., the "endocrine kidneys" were obtained using the technique of Selye and Stone (505) by a partial constriction of the aorta between the origins of the two renal arteries, accomplished by placing the style of a No 22 subcutaneous injection needle alongside the aorta and tying a silk thread tightly around style and vessel. Following subsequent removal of the style, the lumen of the aorta was reduced approximately to the thickness of the wire. The ureter of the "ischaemic kidney" is ligated and sectioned. In 18 rats weighing 260-280 g., the "endocrine kidney" was obtained by the complete ligature of the aorta between the two renal arteries, accompanied

by the ligature and section of the ureter of the "ischaemic kidney". Since the blood pressure changes and the morphological appearance of the "endocrine kidney" were similar, with either technique, rats of both groups are included in table 21. In order to study the chronological changes, the rats were divided into three subgroups :

a) Subgroup Ia : 14 rats, sacrificed at the end of the first week.

b) Subgroup Ib : 7 rats, sacrificed at the end of the second week.

c) Subgroup Ic : 9 rats, killed at the end of the third week.

Group II. Aorta ligature (Ao-lig) : 13 rats of larger size and weighing 340-360 g. with satisfactorily produced small kidney were studied. The aorta was ligated between the renal arteries and the rats were killed at the end of the second week. The ureter was not touched.

The criteria of satisfactorily produced small kidneys or for endocrine kidneys were survival of the renal tissue without macroscopic infarcted zones or hydronephrosis.

In addition, 10 rats weighing from 200-350 g. were used as controls.

All animals were living at the end of the experiment.

At the end of the first, second and third week, blood pressure was measured directly from the carotid artery and recorded on a polygraph (Grass Model 5D).

After macroscopical evaluation, the kidneys were removed and cut in two halves ; one half was fixed in Zenker-formol 10% and stained for juxtaglomerular granules ; the other half served for histochemical studies (Glucose-6-phosphate dehydrogenase (G6PD), 6-phosphogluconic dehydrogenase (6PGD), and α-glycerophosphate dehydrogenase (GPD)).

In some animals, plasma renin activity (PRA) was determined by the micromethod of Boucher et al (504).

b) RESULTS. (Tables 21, 22).

1) Blood pressure.

The blood pressure of the control animals varied from 90 to 135 mm Hg with a mean value of 115 ± 16. We therefore, considered as hypertensive animals those with blood pressure readings above 140 mm Hg.

Group I. EK. (Table 21).

In subgroup Ia, (1 week) 54 % animals became hypertensive. In subgroup Ib (2 weeks) 33% and in the subgroup Ic (3 weeks) 78% were hypertensive.

Group II. Ao-lig. (Table 22).

The percentage of hypertensive animals rose at the end of the second week to 85%.

2) Histological findings.

Juxtaglomerular granulation index (JGI)

Group I. EK. In subgroup Ia, a significant increase ($P < 0.01$) in granularity was found when compared to the control group. This increase is in parallel to the period of ischaemia. At the end of the third week, the JGI was of 68 ± 34.

In contrast to the endocrine kidney, the contralateral kidney showed a significant hypogranularity at the end of the first week (subgroup Ia) JGI = 10 ± 7 ($P < 0.001$). As the period of ischaemia becomes more prolonged (end of third week) the granularity of the juxtaglomerular cells almost totally disappeared (see table 21) in the contralateral kidney.

TABLE 21

GROUP I

Blood pressure (BP), juxtaglomerular granulation (JGI) and glucose-6-phosphate dehydrogenase activity in the macula densa (MDI) in endocrine (L) and contralateral kidney (R).

Sub-group	Duration	No of rats	BP/mm Hg	JGI		G6PD MDI	
				L	R	L	R
Ia	1 week	1	200	40	6	82	46
		2	120	23	2	36	43
		3	120	15	7	38	43
		4	130	50	14	45	56
		5	90	29	11	95	70
		6	180	53	6	86	50
		7	120	41	9	45	61
		8	160	40	6	101	62
		9	---	78	1	---	--
		10	190	30	7	65	62
		11	180	40	24	60	65
		12	190	37	22	75	78
		13	165	36	20	--	--
		14	75	15	12	--	--
Mean ± SD			148 ± 41	38 ± 16*	10 ± 7*	66 ± 23**	58 ± 11**
Ib	2 weeks	1	---	60	2	--	--
		2	135	66	0	106	31
		3	140	7	1	--	--
		4	135	50	5	38	36
		5	100	70	7	74	46
		6	85	76	2	--	40
		7	200	77	1	118	52
Mean ± SD			132 ± 39	58 ± 24*	3 ± 3*	84 ± 6*†	41 ± 8*
Ic	3 weeks	1	140	55	0	--	--
		2	150	44	0	--	--
		3	230	45	1	68	54
		4	180	44	0	75	56
		5	85	110	2	96	65
		6	90	63	0	110	45
		7	150	70	1	125	68
		8	155	46	0	--	--
		9	155	140	0	73	44
Mean ± SD			148 ± 44	68 ± 34*	0.4 ± 0.7*	92 ± 23*†	55 ± 10**

* "p" significant (<0.05) vs control group.
** "p" no significant (>0.05) vs control group.
† "p" significant (<0.05) vs contralateral kidney.

TABLE 22

GROUP II

Blood pressure (BP), juxtaglomerular granulation (JGI), and glucose-6-phosphate dehydrogenase activity in the macula densa (MDI) in ischaemic (L) and contralateral kidney (R) 14 days after complete aorta ligature.

No of rats	BP/mm Hg	JGI		G6PD MDI	
		L	R	L	R
1	200	80	1	67	60
2	180	42	0	84	35
3	160	69	1	123	42
4	210	82	0	79	46
5	90	100	1	70	45
6	145	83	1	--	--
7	180	100	0	62	35
8	170	65	1	64	57
9	170	12	2	60	44
10	180	86	0	83	33
11	145	60	0	38	43
12	150	79	0	31	63
13	100	79	0	37	76
Mean ± SD	160 ± 35	72 ± 24*	0.5 ± 0.6	66 ± 25**†	48 ± 13**

Control values	BP/mm Hg	JGI (10 rats)	G6PD MDI (10 rats)
Mean ± SD	115 ± 16	23 ± 5	57 ± 9

* "p" significant (<0.05) vs control group.
** "p" no significant (>0.05) vs control group.
† "p" significant (<0.05) vs contralateral kidney.

Group II. Ao-lig. A high degree of granularity was encountered in the ischaemic kidney at the end of the second week (JGI = 72 ± 24), and was significantly higher than in the rats of subgroup Ib. At that time granularity almost totally disappeared in the contralateral kidney.

In both groups I and II, interlobular and interlobar arteries of the ischaemic kidneys showed the presence of granular epithelioid cells ; these findings were often observed in the corticomedullary region. However, these arterial granulated cells were not included for the estimation of the JGI. Furthermore, in some instances, small arteries at the hilus of the kidney, presented granular epithelioid cells in the muscularis. Fig. 63 . Even the efferent glomerular arteriole often showed granular epithelioid cells.

"Lacis cells".

The lacis cells in the subgroup Ia of the group I were well preserved and appeared as numerous as in the control group. However, in the subgroup Ib and Ic of the group I and in group II, their number was decreased.

Macula densa cells.

In both groups and even in kidneys with high degree of renal ischaemia cluster of macula densa cells were encountered. However, its morphological pattern is distorted. In subgroup Ia numerous mitotic figures were observed in the endocrine kidney, mostly in the corticomedullary region, and on a lesser extent in the contralateral kidney.

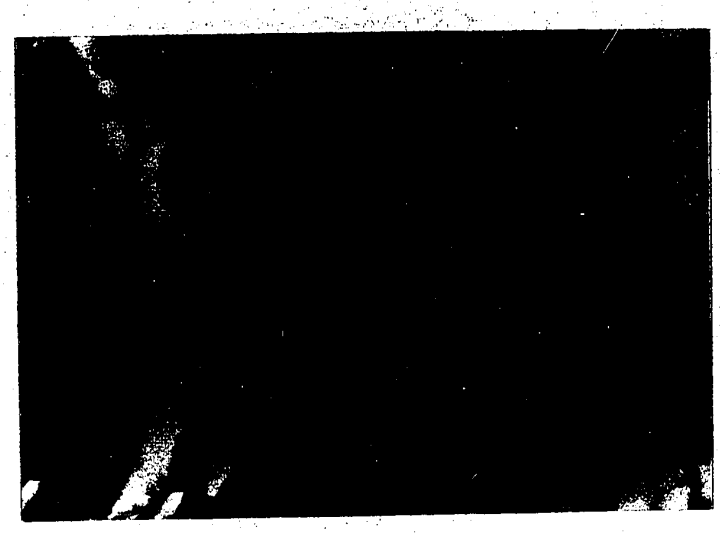
PLATE XXV

FIG. 63. Small artery at the renal hilus showing (in the media) granular cells (Arrows). Left "endocrine kidney". Bowie modified stain. x 500.

FIG. 64. Arteriole showing many granulated cells in the media (Arrow). Two weeks after complete aortic ligature (left kidney). Bowie modified stain. x 500.

FIG. 65. Glucose-6-phosphate dehydrogenase of macula densa (type IV). "Endocrine kidney" after three weeks. Nitro-BT-Co-Ba method. x 670.

PLATE XXV



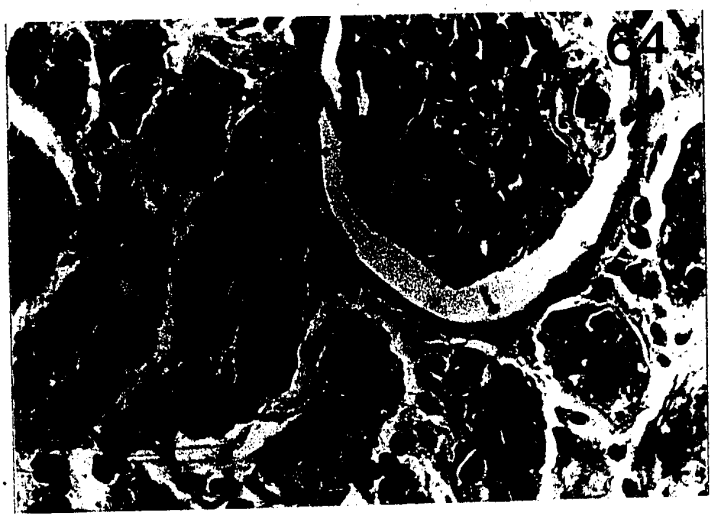
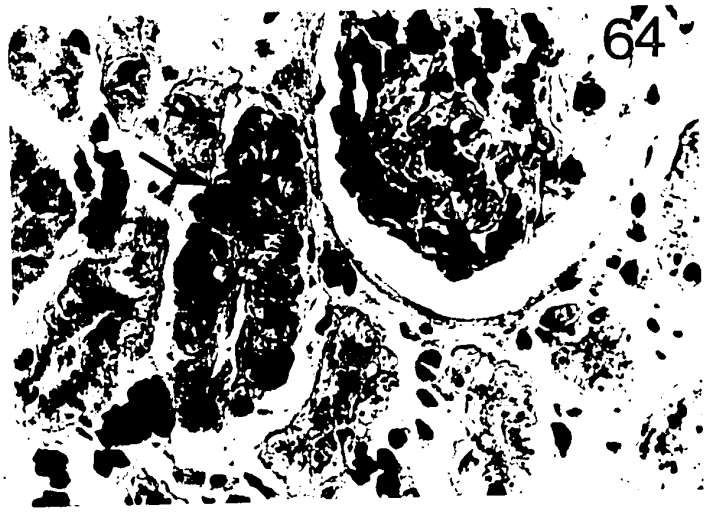


PLATE XXV



3) histochemical findings.

The MDI (G6PD) mean value in control animals was 57 ± 9.

Group I. EK. In subgroup Ia, no statistically significant changes ($P > 0.2$) were found in MDI (66 ± 23) in the endocrine kidney after 1 week of renal ischaemia when compared to the control group. In spite of these findings shown in table 21, 5 animals out of 11, presented values above the upper limit found in control rats. But in subgroup Ib, a significant increase ($P < 0.001$) in the MDI (84 ± 36) was found when compared to the control one. Similar findings (MDI = 92 ± 23) were observed in subgroup Ic.

In the contralateral kidney, the MDI (58 ± 11) of subgroup Ia did not show any significant changes as compared to the control one or to the ischaemic kidney ($P > 0.2$). But after 2 weeks (subgroup Ib) a significant decrease was found (MDI = 41 ± 8) as compared to the control ($P < 0.01$) and to the ischaemic kidney ($P < 0.05$). In subgroup Ic, no significant changes ($P > 0.6$) were found in the MDI (55 ± 10) as compared to the control group, although a significant difference ($P < 0.01$) was observed when compared to the ischaemic kidney.

Group II. Ao-lig. No significant difference in MDI was found in the ischaemic kidney of this group when compared to that of the control group. However, the mean MDI of the ischaemic rats was significantly different ($P < 0.05$) when compared to that of the contralateral kidney.

The 6-phosphogluconic dehydrogenase activity of the macula densa was roughly similar to that of the glucose-6-phosphate dehydrogenase in the ischaemic and contralateral kidneys. In some animals when these

enzymatic activities were increased in the macula densa cells. The afferent arteriole also showed a high activity in these enzymes.

In about the 50% of the isehemic kidneys, the α-glycerophosphate dehydrogenase activity appeared to be increased at the vascular pole of the glomeruli, as compared to the control animals. However, the α-glycerophosphate dehydrogenase and the pentose-phosphate shunt enzymes studied appeared to be increased in the intima and media of the small arteries and arterioles of the contralateral kidney.

Generally, the capsular epithelium of the glomeruli and the corticomedullary region of the ischaemic kidney of both groups showed a high activity of the G6PD and 6PGD activity. However, in the tubular outer cortex (except the macula densa) there was an almost total absence of enzymatic activity. On the other hand, proximal and distal tubules of both peripheral cortex and corticomedullary region of the contralateral kidney, presented high enzymatic activity of the pentose-phosphate shunt enzymes when compared to the control groups.

4) Plasma renin activity (PRA).

Control values of plasma renin activity in control rats are (19 ± 8 ng) (p.120). They correspond closely with those reported by Boucher et al (504) (17 ± 9.5 ng).

In a few rats from both group I and II, plasma renin activity was markedly increased (400, 100, 150, 63, 40, 60 ng) indicating that these "endocrine" or "small" kidneys liberated excess of renin.

c) DISCUSSION.

The experiments described are based on the considerations reported by Selye and Stone (505). If the hydrostatic pressure in the glomeruli

is decreased to a level corresponding to the sum of the osmotic pressure of the blood and the hydrostatic pressure of the urine in Bowman's capsule, then the filtration must cease. The renal artery originates directly from the aorta and splits up rapidly into its smallest branches. Hence, intraglomerular pressure is much higher than capillary pressure elsewhere in the body. This is indispensable for the formation of urine but the ordinary capillary pressure is quite sufficient for internal secretion as shown by the other endocrine organs. This is why we felt that, by total constriction of the aorta between the two renal arteries, to the extent that will decrease capillary pressure in the glomeruli to approximately the same level as normally prevails in other endocrine glands, we would not interfere with the secretory activity of the juxtaglomerular complex but would render filtration impossible. We succeeded in demonstrating the validity of this assumption using the technique employed by Selye and Stone, and the technique of producing "endocrine kidney" by complete constriction of the aorta and ureter ligature in rats from 250 to 280 g. as described in chapter I. However, as shown in the same chapter, animals from 320-400 g. frequently developed hydronephrosis. We also were interested in comparing animals of group I in which urine was not formed, with animals of Group II in which urine is formed in a high percentage of rats.

Our findings contradict the theory (585) according to which hypertension cannot result from a "clamped kidney" if the latter is non-functioning so far as urine excretion is concerned. In spite of the fact that neither non-infarcted zones nor hydronephrosis were observed in the kidneys studied, the percentage of hypertensive animals is higher in the Group II than in the pure "endocrine kidney", although the animals in

Group II were older. It is possible that age might well be an important factor to consider.

We found in a number of animals eliminated from these experiments, that their blood pressure was normal or slightly increased when they presented hydronephrosis. It is of interest that Selye and Stone did not observe any excessive proliferation in the juxtaglomerular cells of the "endocrine kidney" and expressed the possibility that the tubular cells were responsible for the endocrine production of renin. It is to be noted that these authors did not use any special stains for demonstrating juxtaglomerular granules.

In our experiments a marked hypergranularity was encountered in spite of the absence of urine formation and of the fact that no intratubular fluid reached the macula densa, since the tubular lumen is replaced by tubular cells proliferation. These findings provide convincing proof that the tonicity or osmolarity of the tubular urine at the level of the macula densa is not essential for the stimulation of juxtaglomerular cells granularity.

The presence of granular epithelioid cells in the interlobar and interlobular arteries and less frequently in the small arteries of the renal hilus, gives the special capacity to the smooth muscle cells of the small renal arteries to be transformed into these granular cells. It is obvious that, in such granular cells, the role of the macula densa must be ruled out. The presence of granular cells in the efferent arteriole adds further evidence in favor of considering such arteriole as a part of the JG complex.

Comparable experiments were those of Tribe and Heptinstall (214). These authors produced scars in rat kidneys by experimental pyelonephritis and localized traumatic lesions to the renal papilla in order to produce regions of tubular atrophy and loss with persistence of the glomeruli. JG granules were demonstrated in both these types of renal scars and increased when the rats were subjected to bilateral adrenalectomy, with sodium supplement.

The experiments by Tribe and Heptinstall (214) and our results are further evidence for the stretch receptor theory, as stimulus of the JG cells, although the possible influence of other stimuli such as the plasma volume, humoral factors or nervous system cannot be ruled out.

The low or absent JG cells granularity in the "untouched kidney" in rats which failed to develop hypertension is difficult to explain if we consider degranulation as a consequence of an increased perfusion pressure. A possible explanation is the high angiotensin levels (in spite of the blood pressure levels of the animals) from the ischaemic kidney suppressing renin synthesis in the contralateral one.

In addition, angiotensin injections were shown to produce degranulation of the JG cells. Even with this possibility, we cannot explain why these rats did not develop any increase in blood pressure.

Our results in the "endocrine kidneys" after 3 weeks of ischaemia establish a parallel relationship between the JGI and MDI. Similar results were reported by Fisher in the clipped kidney of rats, after 4 weeks of renal ischaemia. (140).

The findings reported by Hess and Pearse (322) and Hess and Regoli (157) require special comments. Hess and Pearse studying the chronology

of renal changes in clipped kidneys of renal hypertensive rats showed after 3 days of renal ischaemia normal activity of the G6PD in one animal, and increased in another. The G6PD in the untouched kidney was normal. After 18 days the G6PD was increased in all ischaemic kidneys, but was normal in the untouched kidneys in about the half of the animals. Later, Hess and Regoli (157) reported in the same type of experiments (in relatively few animals) that after 5 days an increase of the G6PD in the macula densa usually occurs in the ischaemic kidney accompanied by a decreased activity in the same cells of the untouched kidney. They suggested that the renin formation appeared to be unrelated to the changes of the G6PD activity and not to the JG cells granularity.

Our results during the two first weeks of ischaemia demonstrate that no correlation exists between the JGI and MDI, between MDI and PRA, or between the three parameters studied and the blood pressure. Furthermore, according to Fisher, (140), G6PD activity in the macula densa appeared to be mostly related to the degree of ischaemia as reflected by atrophy of the ischaemic kidney. In support of this observation are the results obtained showing that the degree of kidney atrophy is much more marked in the "endocrine kidney" (Gr. I Ao-lig + ureter ligature) after 2 weeks of renal ischaemia that in the simply ischaemic kidney (Gr. II Ao-lig), and correspond to G6PD activity when both groups are compared at the same experimental period.

Our failure to observe a significant decrease in the enzymes studied in the contralateral kidney in most of the animals studied does not necessarily mean that our observations are in direct conflict with those of

other authors since the degree of lesions in the small vessels of the untouched kidney is much more pronounced following our aortic ligature procedure than that of the clipped renal artery. It should be recalled that Hess and Regoli pointed out the increased G6PD activity in macula densa cells when the arteriolar lesions in the untouched kidney are very pronounced.

Since no relationship of the MDI with JGI or PRA was found, various possibilities could be suggested for the role of the macula G6PD in such experimental conditions. A possibility could be that since it appeared to be related to the degree of ischaemic atrophy, a relative degree of ischaemia by itself could increase the hexosemonophosphate pathway in the macula densa. Such findings were reported by Vagner (583) during the initial period of acute myocardial infarction in man. Bing's group (584) has also described in dogs that following myocardial infarction, the HMP enzymes and the rate of lipid synthesis were increased in the affected areas.

B) ELECTRON MICROSCOPIC STUDIES OF THE JUXTAGLOMERULAR COMPLEX (JGA) IN ISCHAEMIC AND CONTRALATERAL KIDNEYS OF RATS WITH RENOVASCULAR HYPERTENSION.

Since there is no marked difference by light microscopic studies in morphological changes of the JGA in kidneys of rats with hypertension induced by unilateral renal artery constriction as compared to those obtained by the aortic ligature between the origin of the two renal arteries, it appeared of interest to extend the studies to the ultrastructures of the JGA as seen by the electron microscope. This section deals with hypertensive rats with "clipped" kidneys.

a) MATERIAL AND METHODS.

Severe ischaemia was produced in 20 male Wistar rats weighing 200-250 gr. by placing the style of a No 24 or 25 subcutaneous injection needle alongside the left renal artery and tying a silk thread tightly around the style and vessel. Following subsequent removal of the style the lumen of the artery was reduced approximately to the thickness of the wire. Blood pressure was recorded by tail plethysmography and five hypertensive rats were chosen for this investigation.

The experiment was ended at the end of the second month after renal arterial constriction. The fixation was accomplished in vivo by perfusing 1.5% buffered (mono-dipotassium phosphate M/10) glutaraldehyde, through the aorta, and by dripping cold buffered osmium tetroxide 2% on the kidney's surface. Following the procedure described in chapter I, the thin sections were stained with Reynold's method. In addition, three other rats submitted to the same experimental protocol served in order to analyze their JGI in the ischaemic and contralateral kidneys. The fixation and staining procedures for demonstrating juxtaglomerular granules was performed as previously described.

b) RESULTS.

JGI. The values of the ischaemic kidneys of the three animals studied were 58, 67 and 70 with almost total absence in the contralateral kidney.

Juxtaglomerular cells.

Ischaemic kidney. An increase in the granularity and size of granular cells appeared to occur. In some instances, light and dark cells were seen in the media of the afferent arteriole. In the light cells the difference in electron density seems to be related not only to lesser con-

PLATE XXVI

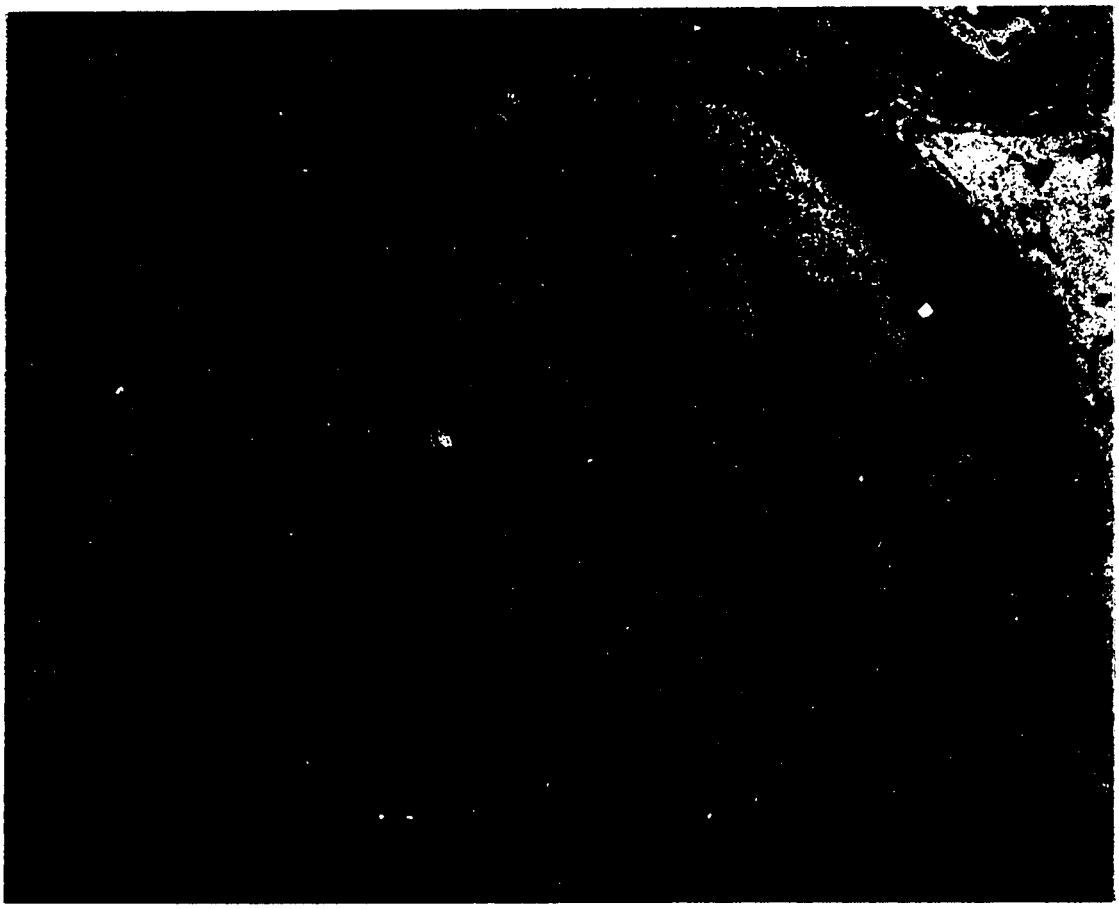
FIG. 66. Glomerular arteriole (cut obliquely) showing light granulated cells (LG) and dark (DG). Arteriolar lumen (Lu). Clipped rat kidney after two months. x 4 500.

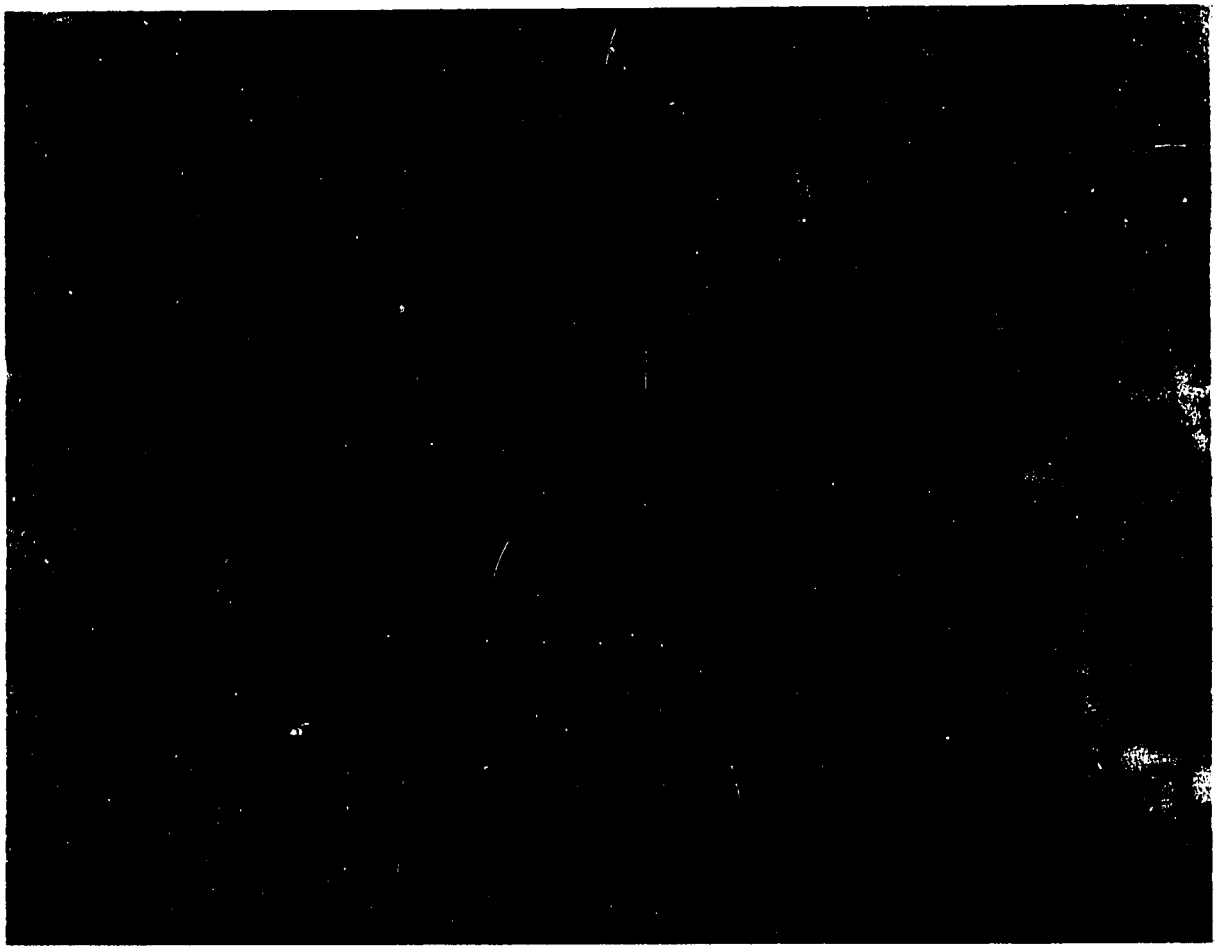
FIG. 67. Electronmicrograph showing continuation of the endoplasmic reticulum cisterna content with the matrix granules (Arrows). Clipped rat kidney after two months. x 50.000.

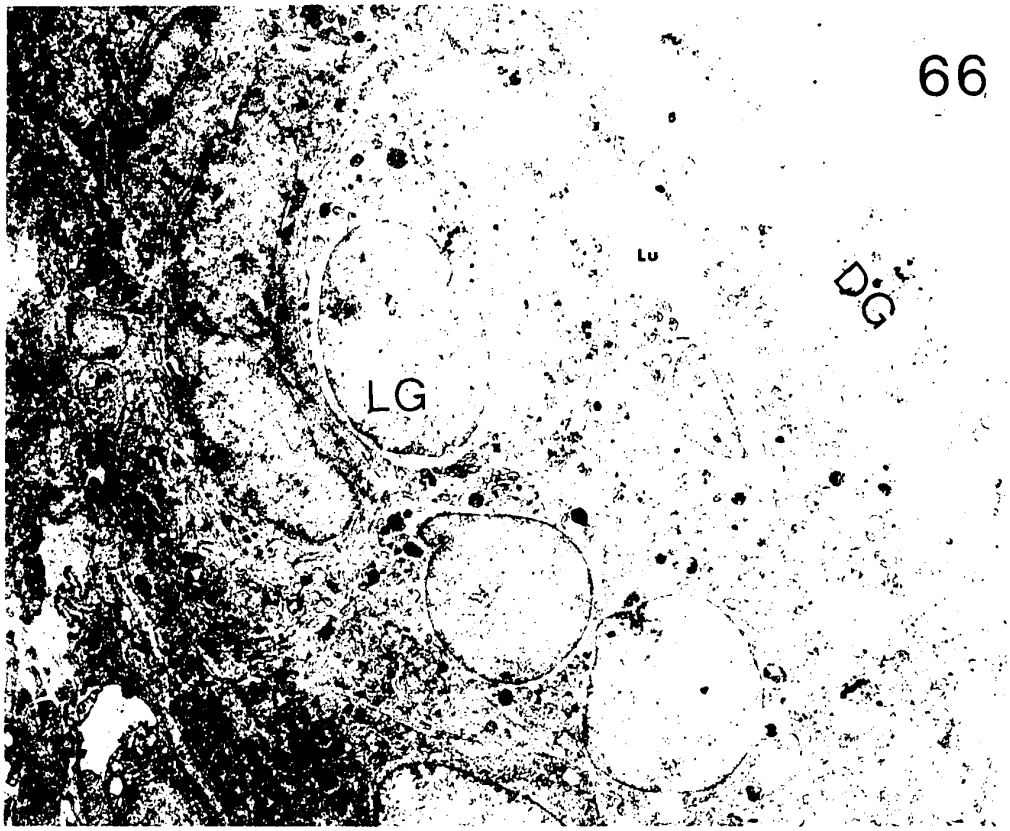
PLATE XXVII

FIG. 68. Electronmicrograph showing the Golgi apparatus (Go) of a granular cell from a clipped rat kidney after two months. Granules (G). Nucleus (N). x 50 000.

FIG. 69. Electronmicrograph showing a vacuole-like formation (see text) in a granular cell. Note the ovoid light bodies making digitations into the vacuolar lumen (Arrow). Clipped rat kidney after two months. Endothelium (Ed). x 17 500.






66
Lu
DG
LG

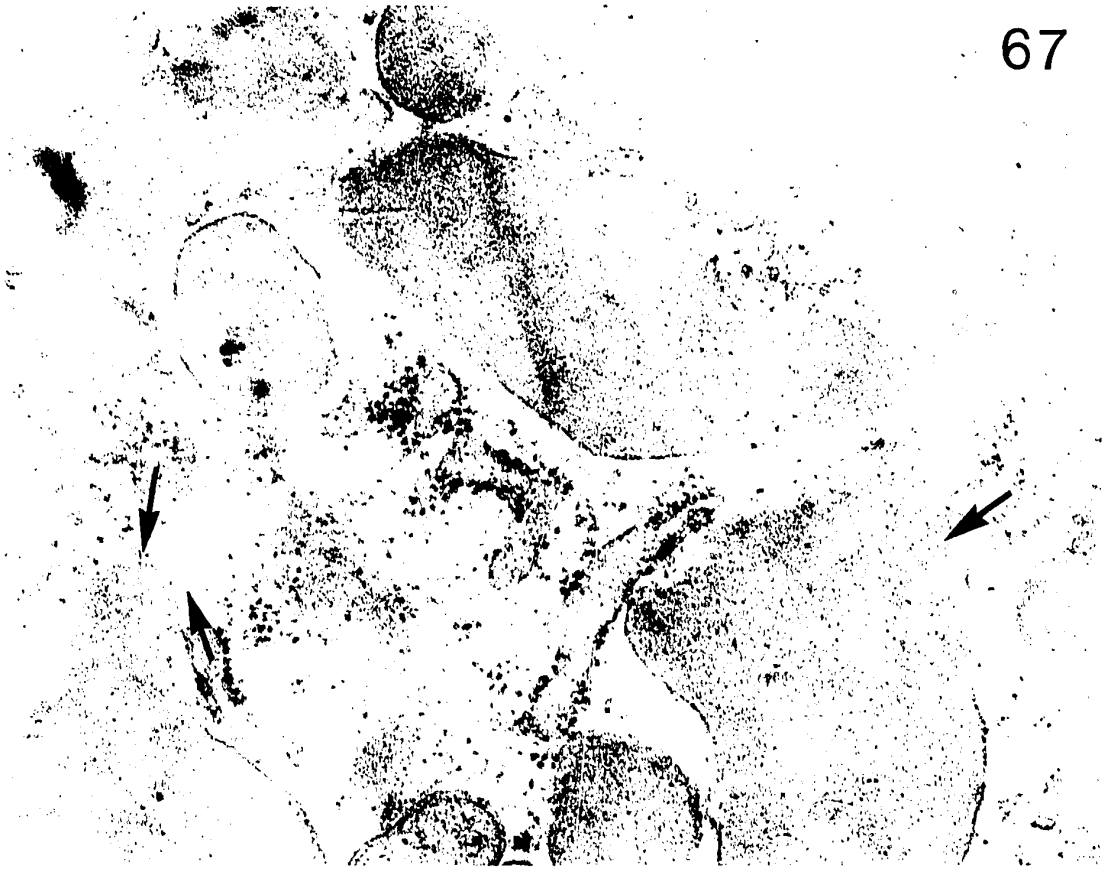

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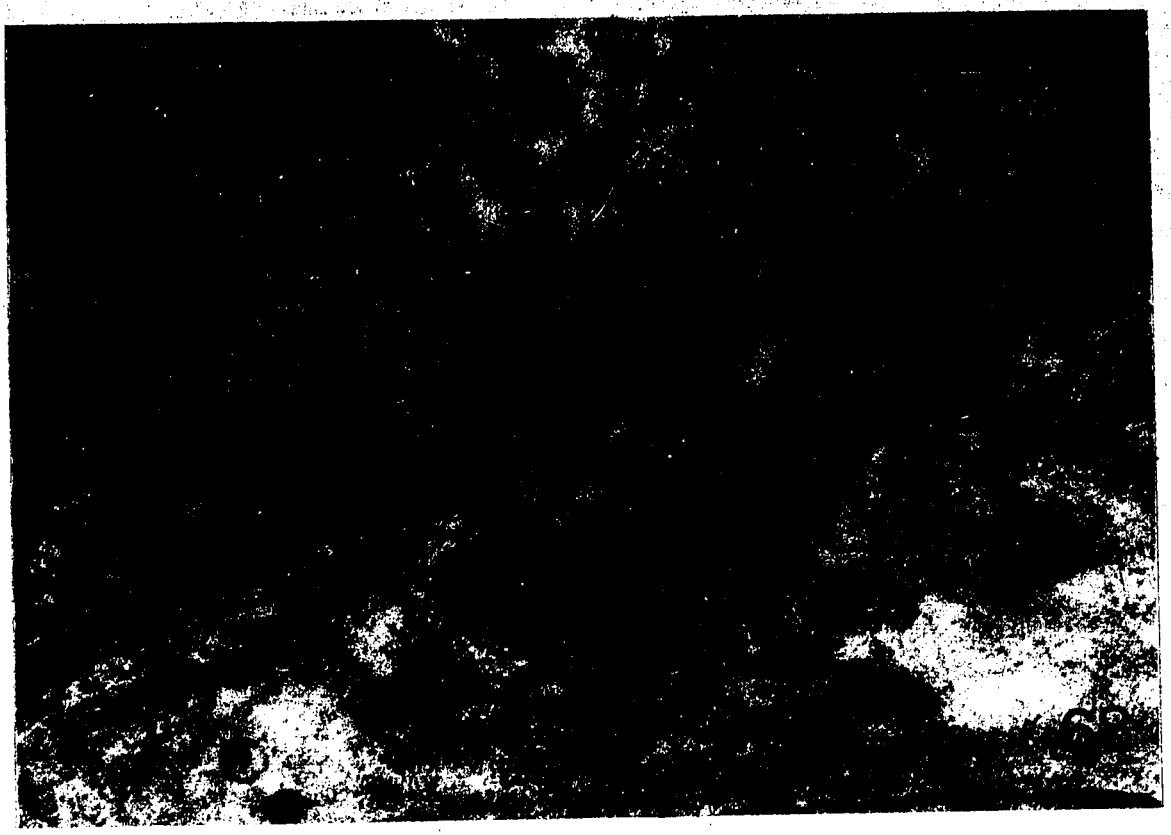
PLATE XXVII



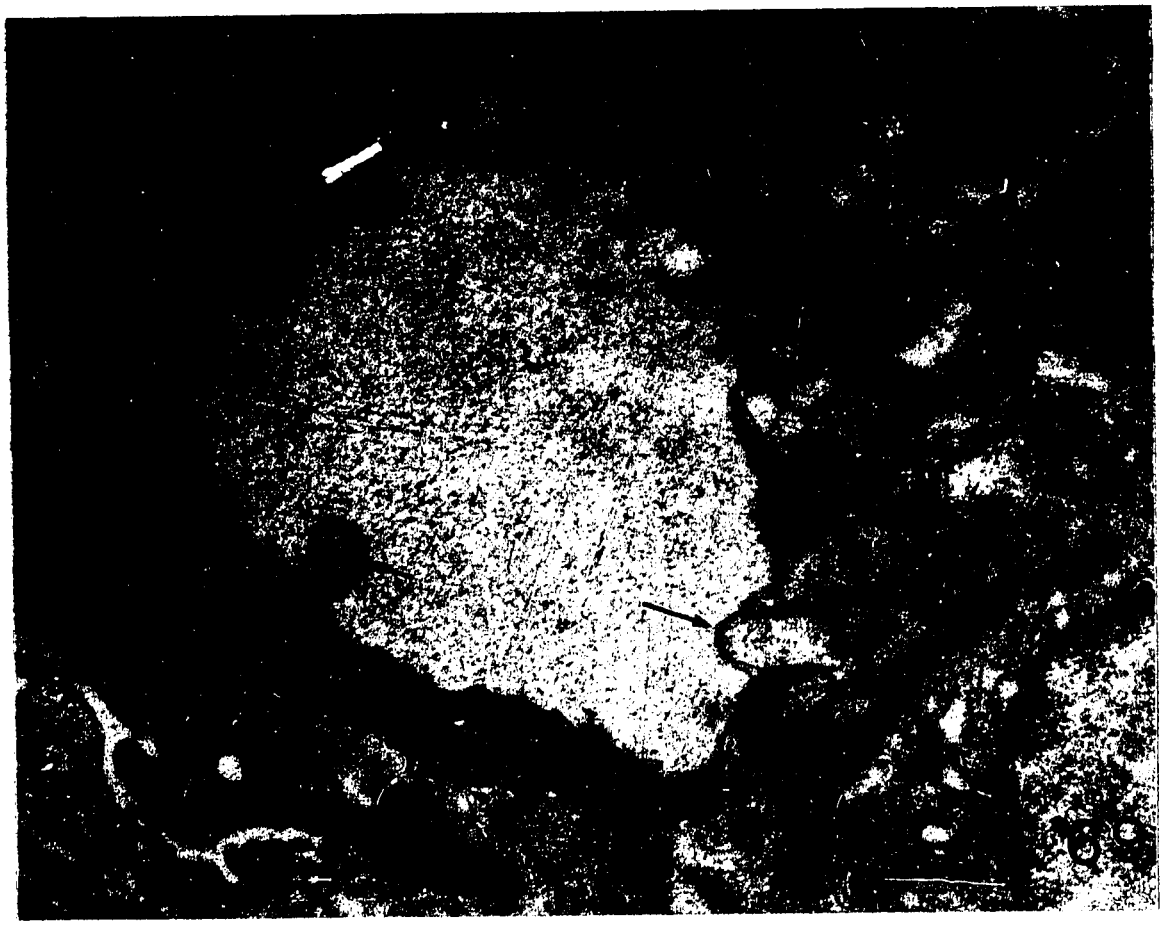
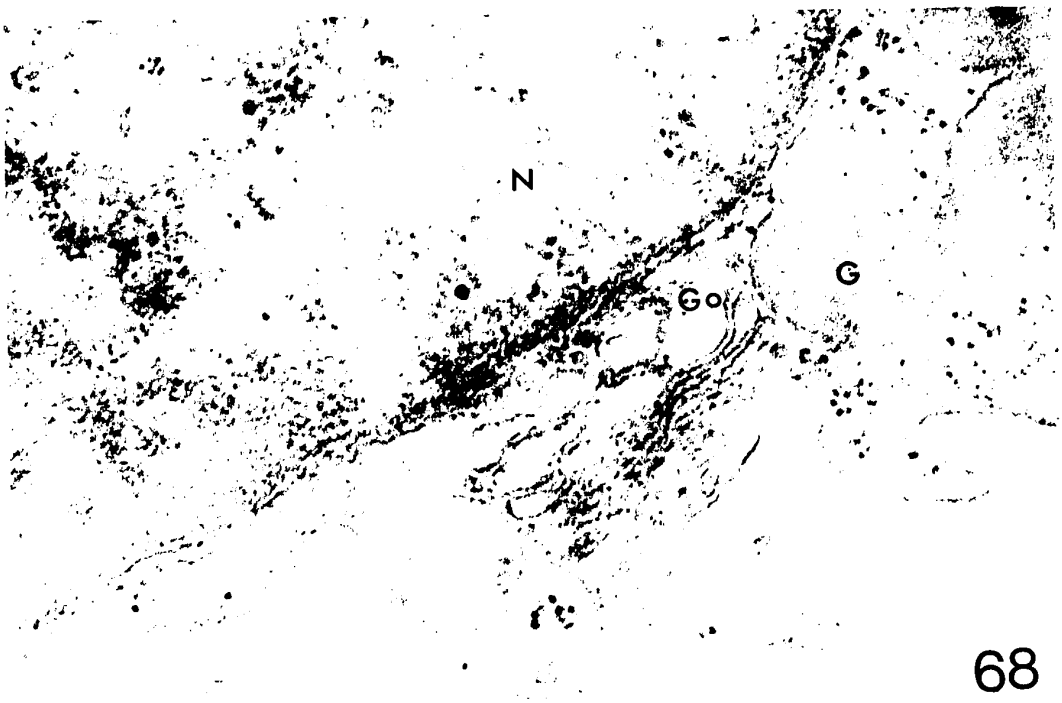


PLATE XXVII



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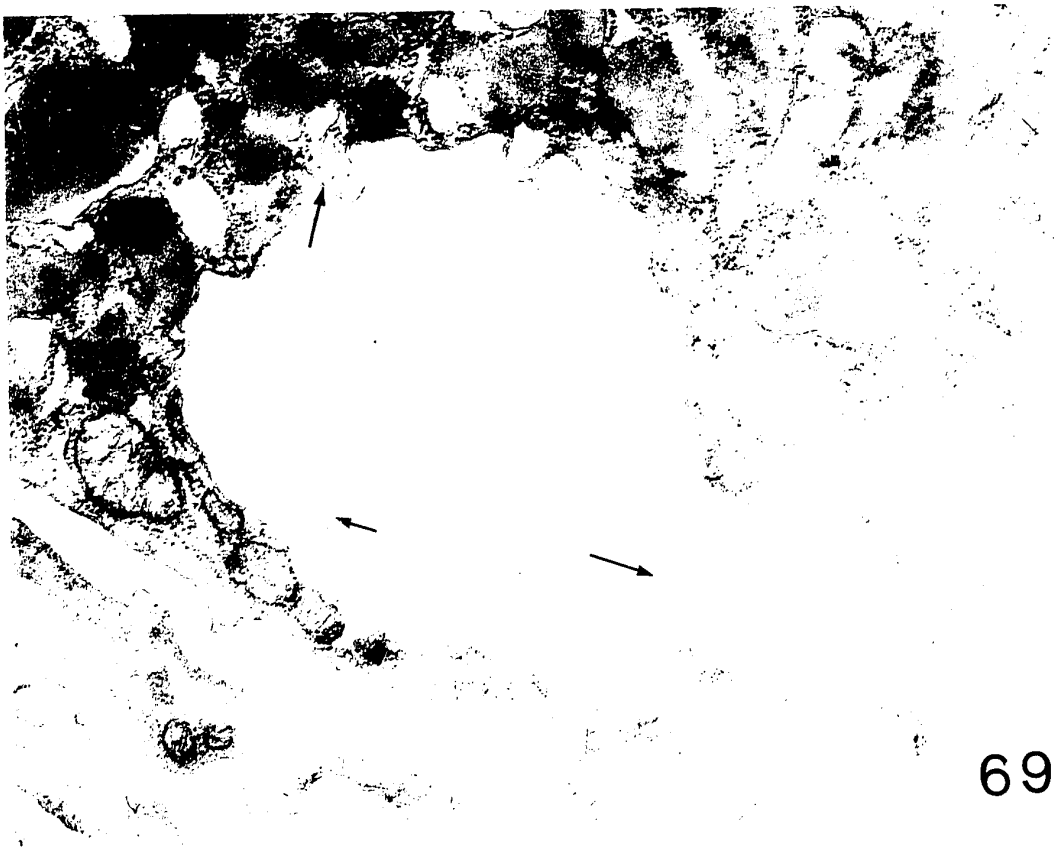

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centration of organelles, but also to a lighter cytoplasm between them. The light cells (Fig. 66) are filled with a few mitochondria, the number of granules appeared to be less numerous than in the dark granulated cells. However, their morphological characteristics were similar. The endoplasmic reticulum was moderate. The Golgi apparatus was prominent, and no myofilaments were seen.

The dark granulated cells had similar characteristics as those described in sodium-restricted rats. However, endoplasmic reticulum generally exhibited more considerable dilatation and its cisternae contained material which had greater electron opacity in the ischaemic kidney. As mentioned in chapter IV, in the formative stages of some of the granules, the plane of section passed through distended endoplasmic reticulum where it was directly continuous with the limiting membrane of a granule (Fig. 67) or where its contents were confluent with the matrix of a granule.

The lamellar sacs and the small vesicles of the Golgi apparatus (Fig. 68) seem to be as numerous as in sodium-restricted rats. Myofilaments and microspheres were a common finding. Occasionally some vacuole-like formations were found (Fig. 69). However, they were limited by a double membrane and light round bodies impinging upon it. They contain a flocculent material of the same density as the vacuole-like formations.

Contralateral kidney. An almost total absence of granular cells occurs in the afferent arteriole, and the medial layer is formed exclusively by smooth muscle cells (Fig. 73).

Lacis cells.

No major differences in the lacis cells of the ischaemic and contra-

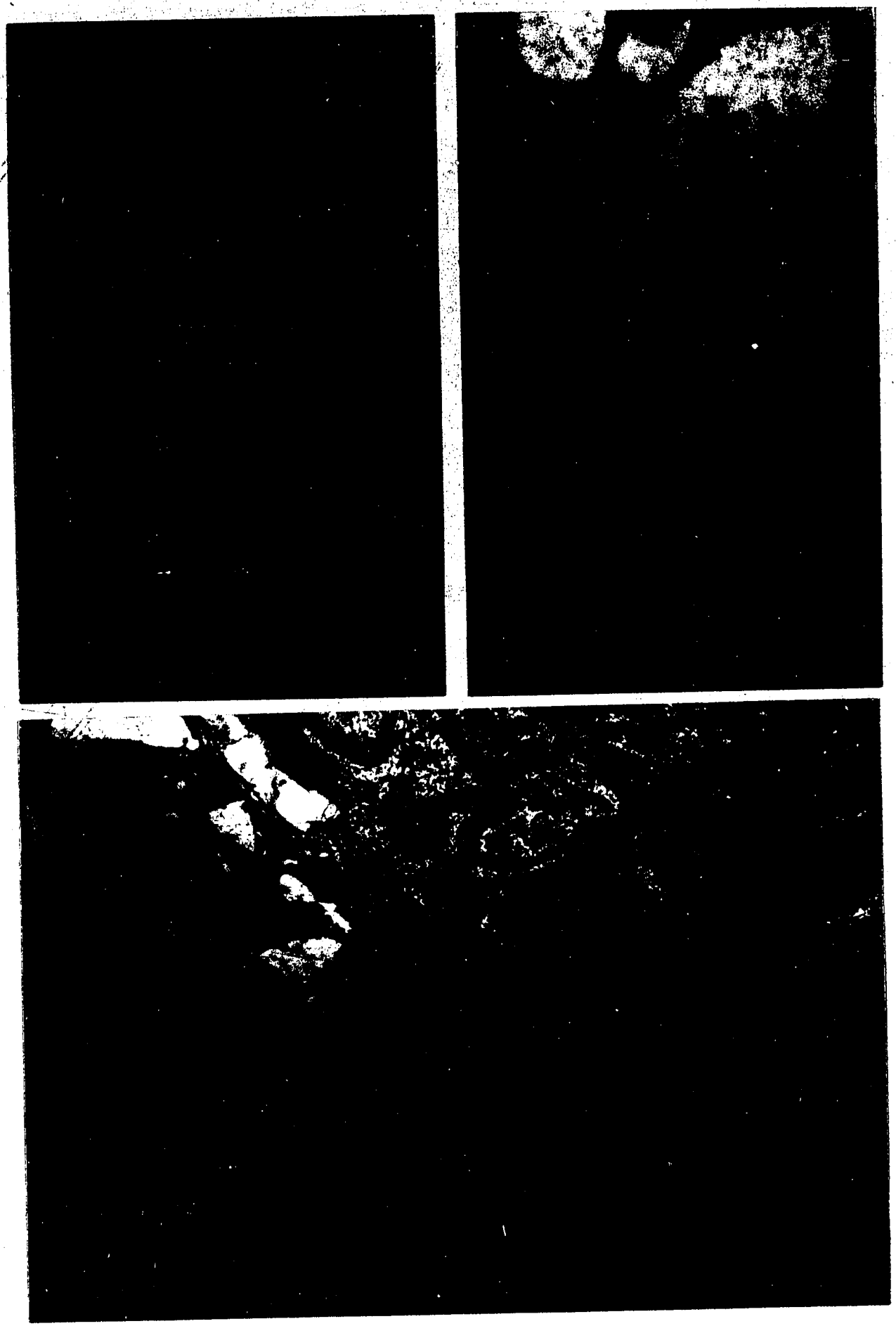
PLATE XXVIII

FIG. 70. Macula densa cell (MD) from a kidney with severe ischemia (after 2 months of clamping). Note the few mitochondria present and the large sub-basilar compartments. Granular cell (GC). x 4 250.

FIG. 71. Serial section from the upper region of the macula densa cell of fig. 70 showing a basal Golgi apparatus (Large arrow) and the relative abundance of endoplasmic reticulum and ribosomes (Small arrow). Nucleus (N). Mitochondria (M). x 17 500.

FIG. 72. Electronmicrograph of a rat kidney with severe ischemia (after two months) showing the macula densa cells (MD). The arrows point to lipofuscin-droplets in the macula densa. Note the absence of granules in the lacis cells (LC). Granular cells (GC). x 7 500.

PLATE XXVIII



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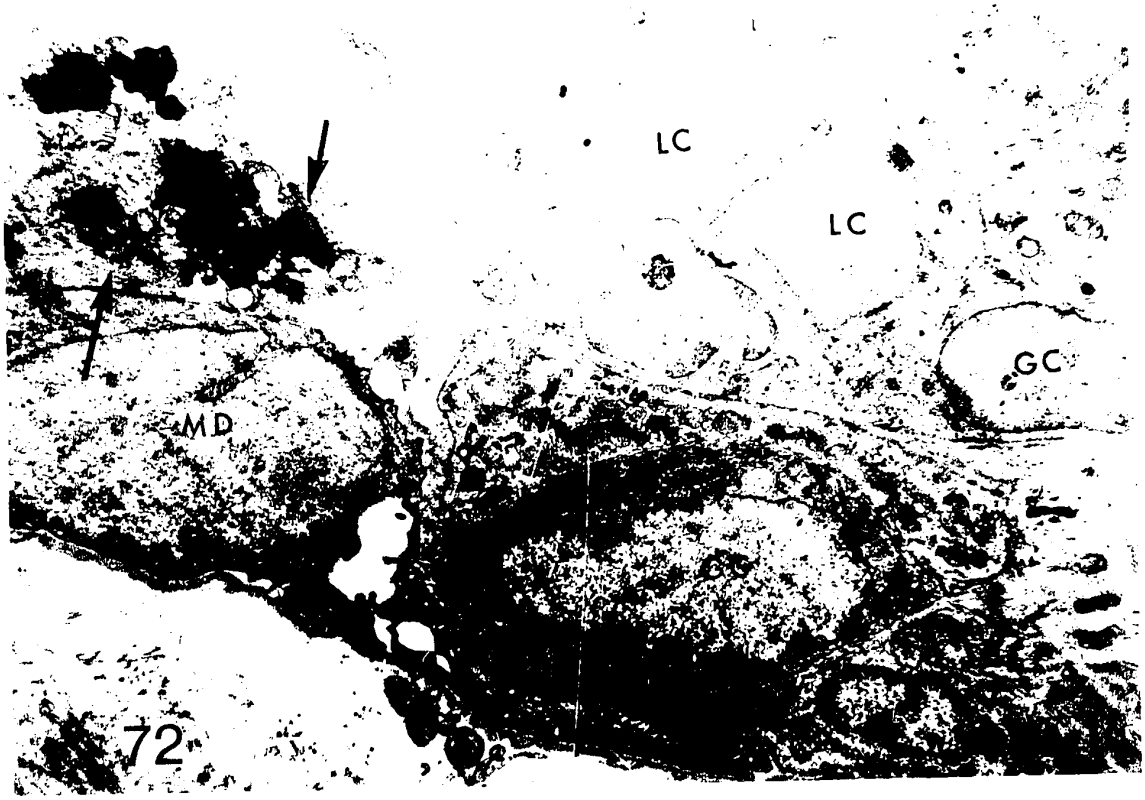

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lateral kidney were seen when compared to those described in chapter IV.

Macula densa.

Ischaemic kidney. In some instances, some distorted cluster of macula densa cells were found (Fig. 70). The nuclei are sometimes irregular. The cytoplasm has few mitochondria in the cell and some of them appeared to be swollen. Lipofuscin droplets mostly located in the basal region were often found (Fig. 72). The Golgi apparatus was localized in the basal region. In some sections, the amount of endoplasmic reticulum and free ribosomes appeared numerous. The infolded membranes at the base of the cells were practically absent and the extracellular or sub-basilar compartments (Fig. 71) were numerous and enlarged. Macula densa cells digitation (Fig. 72) bordering granulated cells were observed in some planes of section. Nerve fibers were also found near the macula densa and granular cells.

Contralateral kidney. The most striking feature observed quite often was the presence of macula densa cells in some plane sections with different electron density (Fig. 73). The light electron dense cells differ mainly from the dark electron dense cells not only in possessing a lighter cytoplasm but also in their mitochondria which appeared to be less numerous than in the dark cell. A basal Golgi apparatus is illustrated in a light cell in fig. 74 and, a paraluminal location of the Golgi apparatus is shown in a dark cell in fig. 75.

The endoplasmic reticulum is more developed in the dark cell than in the light cells.

The basal membrane of macula densa was clearly limited, infolded membranes are very few as compared to the opposite cells of the same part

PLATE XXIX

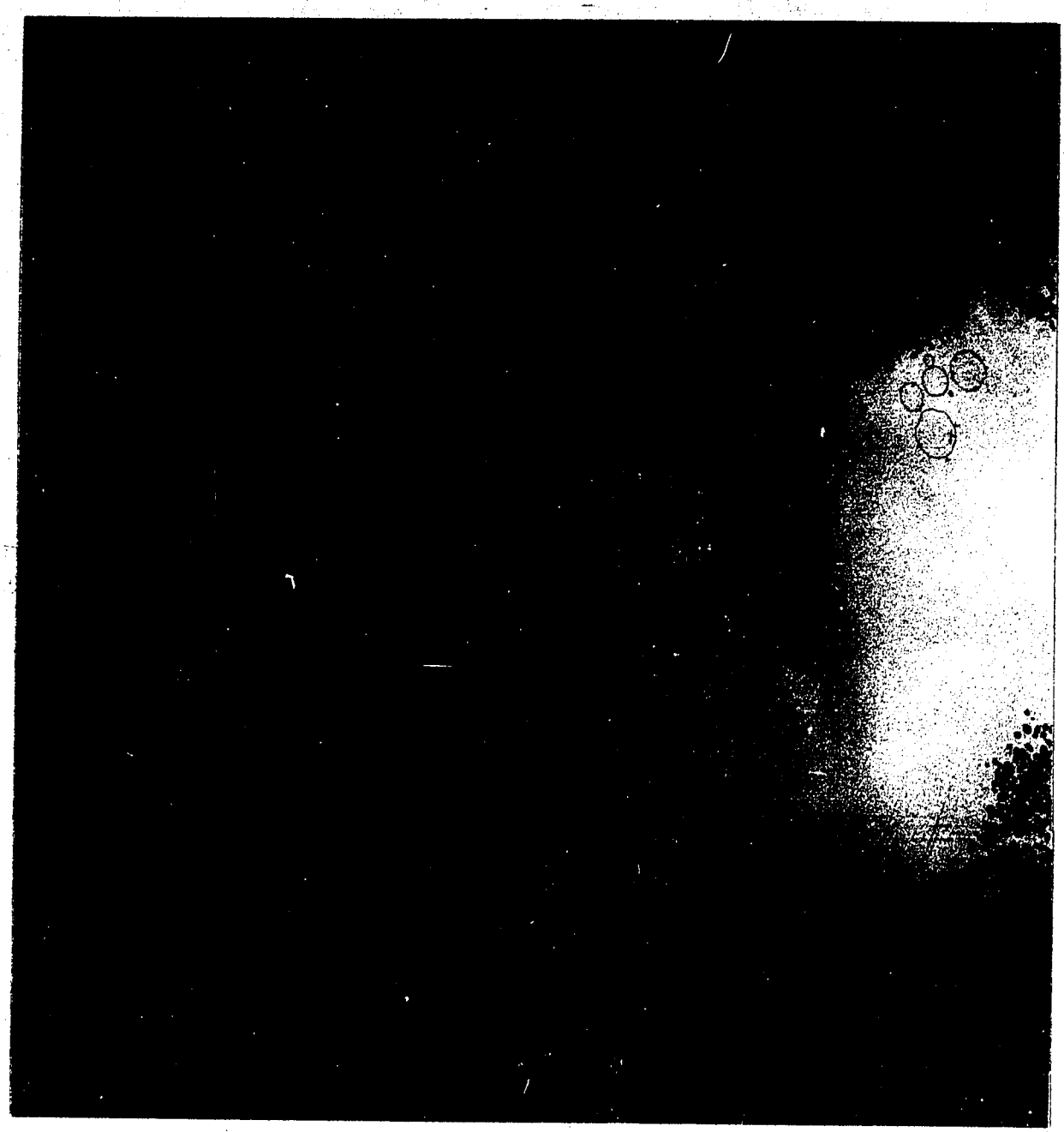
FIG. 73. Untouched kidney of a clipped hypertensive rat. Topographic view showing : a glomerular arteriole (GA) in which there are only smooth muscle cells (SM), lacis cells (LC), macula densa (MD). Note the presence of light macular cells (LM) and dark (DM). Note also the irregular distribution of the mitochondria and the near absence of infolding membranes in the macula densa. The opposite side of the distal tubule shows a regular pattern of mitochondria and infolding membranes . x 4 250.

PLATE XXX

FIG. 74. Higher magnification of fig. 73, showing light macular cells (LM) and dark (DM). Note the small mitochondria of the light cells. The arrow points to the Golgi apparatus of a light cell. Note the prominent nucleolus of the dark cells. x 7 500.

FIG. 75. Higher magnification of the upper right portion of fig. 73 showing the paraluminal localization of the Golgi apparatus (Arrows) of a dark cell. Tubular lumen (Lu). x 11 500.

PLATE XXLX



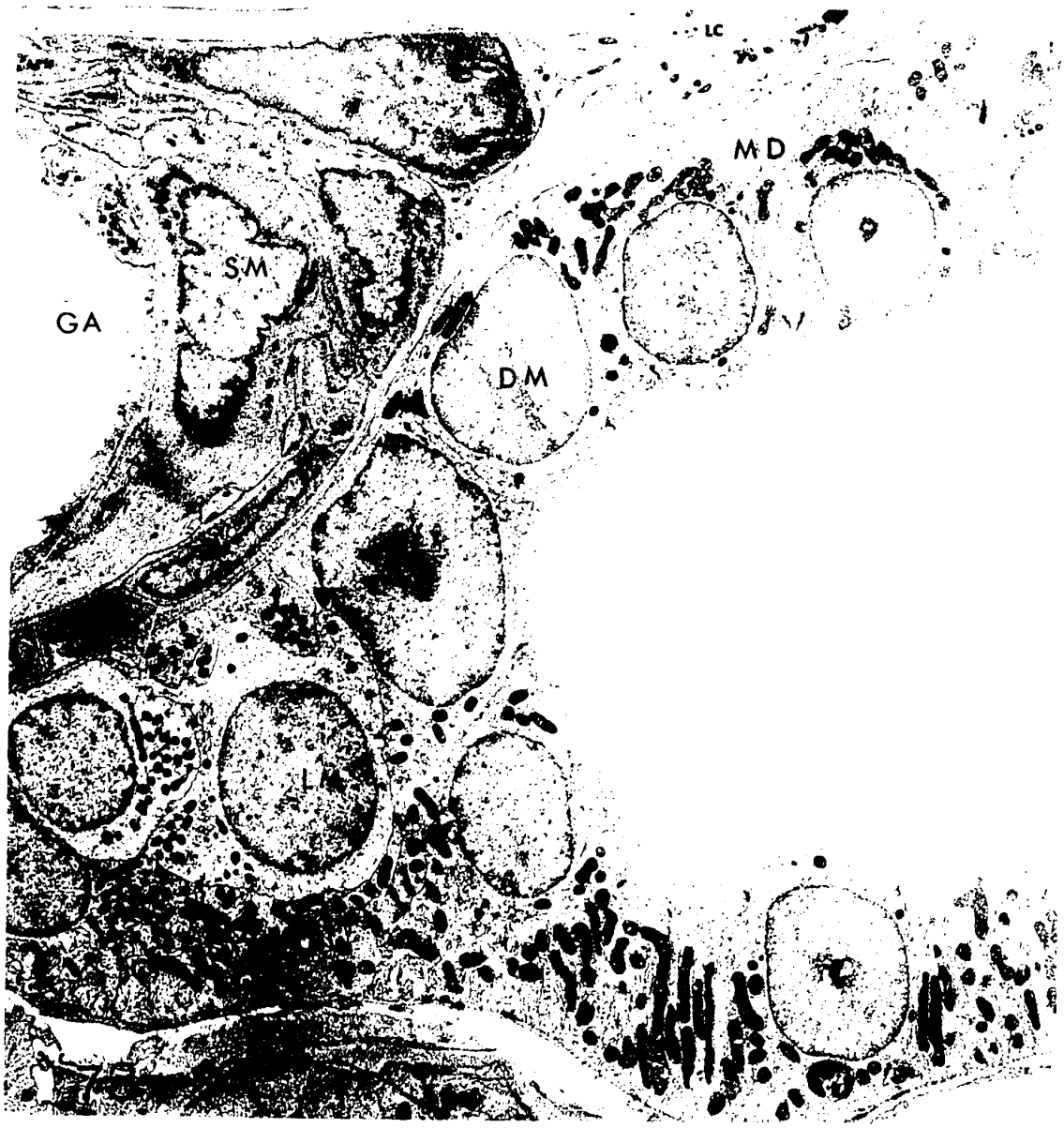

LC
MD
SM
GA
DM

PLATE XXX

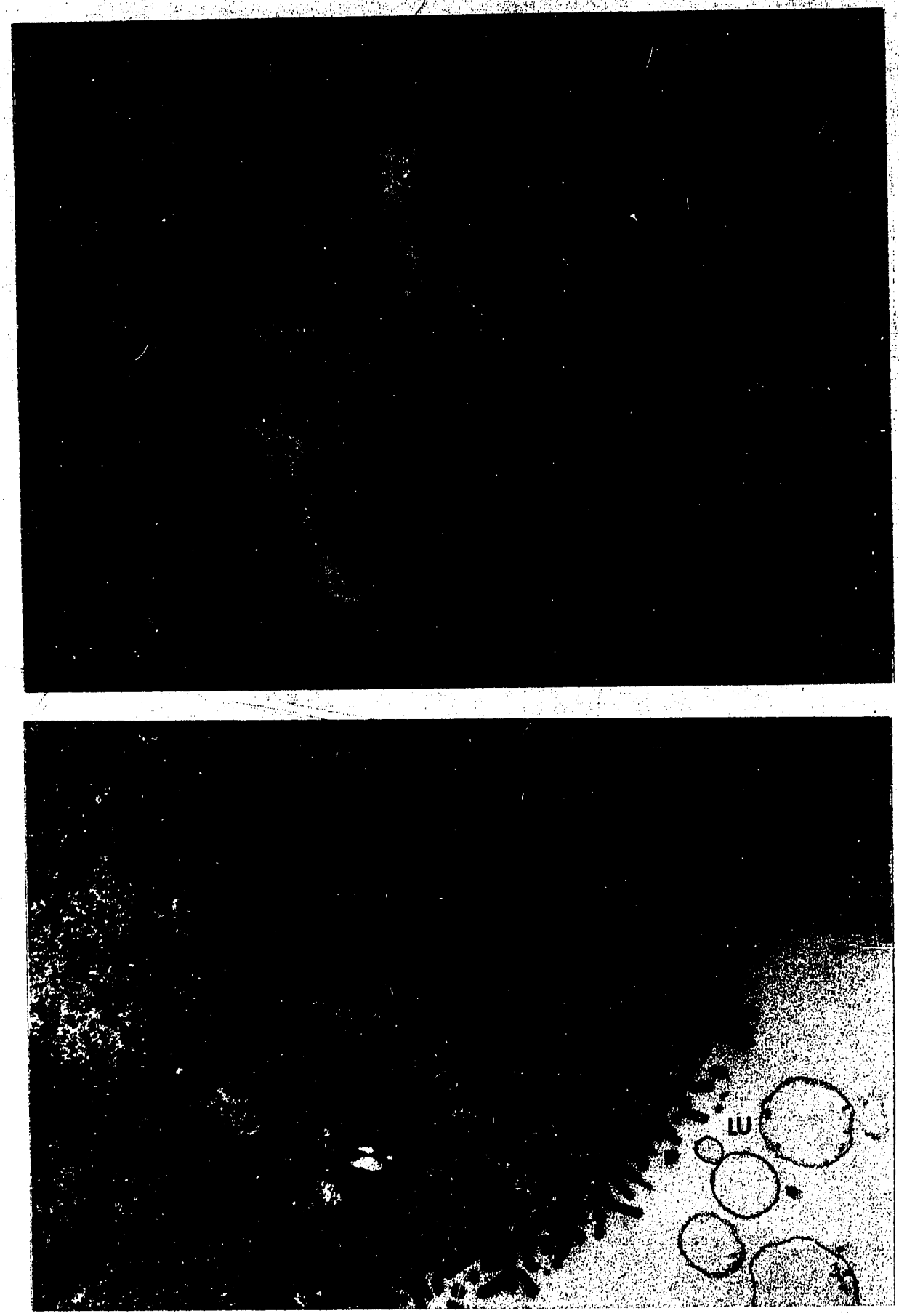
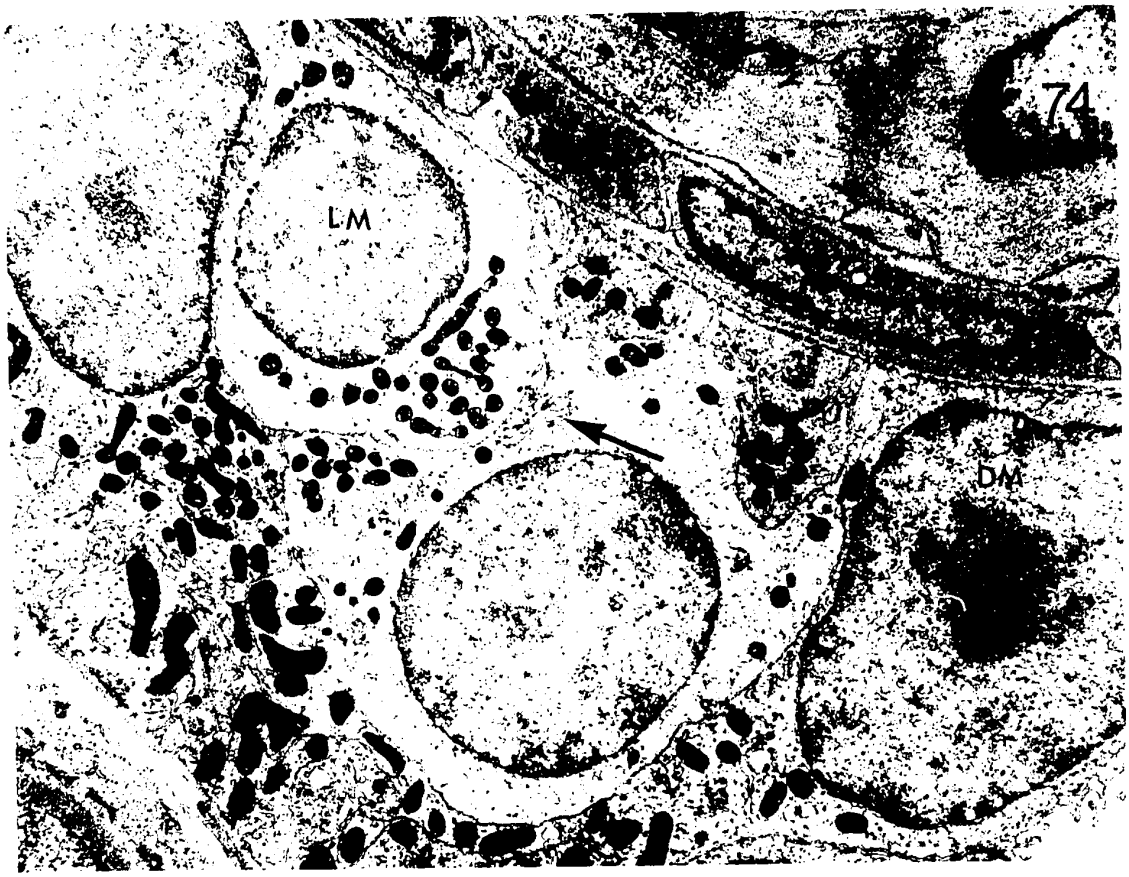
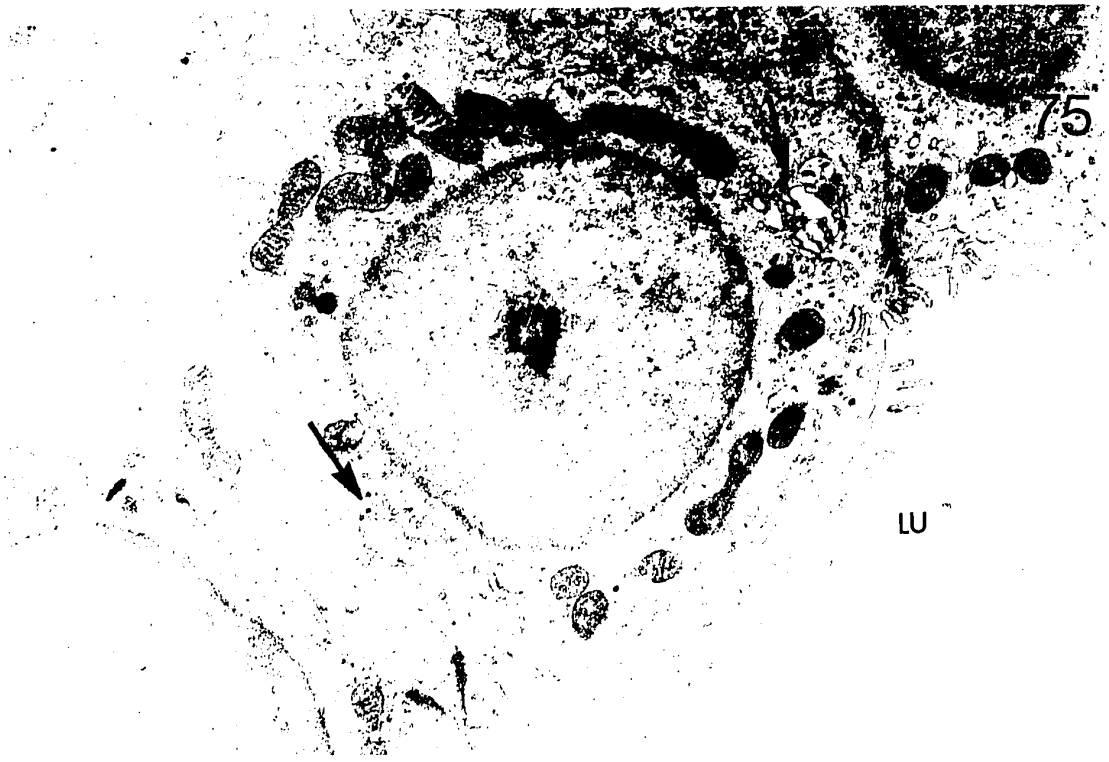


PLATE XXX





of the distal tubule.

c) DISCUSSION.

Light microscope studies of ischaemic kidneys show an increased granularity of the juxtaglomerular cells.

Few electron microscopic studies have been done in the juxtaglomerular apparatus of the ischaemic kidneys of rats and most of them have been related to the juxtaglomerular granulated cells.

The light granulated cells in the ischaemic kidney resemble those clear swollen hypergranulated cells described in ischaemic kidneys in rabbits by Hatt et al (603). Furthermore, Hatt et al (352) have shown in rats treated with DCA and sodium chloride the same type of cells, although with a much lesser degree of granulation. They suggest that the lighter cytoplasm could be due to cell edema.

Since the general morphological features of the dark cells in the ischaemic kidney are essentially the same as those described in sodium-restricted rats, only some features will require further comments. Barajas and Latta (16) have suggested that cells with few granules tend to have a large Golgi apparatus and conversely cells with many granules to have a small Golgi complex. Our observations with serial sections do not support this view, neither in sodium-restricted rats, nor in the ischaemic kidneys, in which several hypergranulated cells have been shown with prominent Golgi systems.

In the granular cells of the ischaemic kidney, the relationhip of endoplasmic reticulum to some granules occurs to a greater extent than in sodium-restricted rats. Direct transfer from endoplasmic reticulum to granules is highly suggested (Fig. 67).

The vacuole-like formation indented by the light ovoid bodies can well fit with the light microscopical observations that we have described in chapter I. However, due to the presence of a double membrane limiting the cavity it is tempting to consider them as possible cytolysosomes. Similar figures as those already described have been also found by Hatt et al (603) in the juxtaglomerular granular cells of the rabbit ischaemic kidney.

Our research work in rats does not support the suggestion of the authors (37) concerning the transformation of the lacis cells into granulated cells. However, we cannot completely rule out this possibility in other animal species, or under other experimental conditions.

The presence of such large sub-basilar compartments in the macula densa, the basal Golgi apparatus and the thin basal membrane in some places suggest that some tubular fluid could pass to the juxtaglomerular or to lacis cells. However, the same kind of compartments were found in other parts of the nephron in the ischaemic kidney. It appears therefore, that these alterations in the ischaemic kidney are not specific for the macula densa. Furthermore, the lipofuscin droplets were also encountered in other parts of the distal tubule. It is difficult to say if the endoplasmic reticulum is quantitatively increased as compared to that of the control animals. In some sections, the endoplasmic reticulum appears to be much better developed than in control or sodium-restricted rats, even if the mitochondria are not increased in number. It is very hard to understand that the increased activity of the glucose-6-phosphate dehydrogenase of the macula densa cells of the ischaemic kidney is only related to the localization of this enzyme in the mitochondria as suggested by Hess and Pearse (322). The amounts of dot-like formazan deposits

shown in their figures do not correspond in any instance to the amount of mitochondria reported in the electron microscopy studies. It is tempting to suggest that the location of the hexosemonophosphate shunt enzymes might well be located in the mitochondria as well as in the microsomes as it has been found by other authors (297-300). Since we failed to observe in the ischaemic kidney quantitative or qualitative changes in the mitochondria of the macula densa it is highly suggestive that the localization of the enzymes of the hexosemonophosphate shunt might be associated with the microsomes. The increases in lipofuscin droplets in the macula densa may be related to the increase of glucose-6-phosphate dehydrogenase found after renal ischaemia.

Contralateral kidney. Since no granules were observed in the afferent arteriole of the glomerulus, and only smooth muscle cells were encountered it may be possible that an inverse process to that described in chapter IV (section B) can occur.

No explanation can be advanced for the light macula densa cells observed. However, coincident with our observations are the light microscopic studies in man and dog by de Muylder (281, 282) who has described the presence of light and dark cells in the macula densa. In addition, Hatt et al (285) presented electronmicrographs showing light macula densa cells in rats treated with DCA and sodium chloride. Both authors interprete these findings as representing an edematous state of the cells. A curious finding was the location of the Golgi apparatus, sometimes in the parabasal region, and in other cells in a paraluminal location (as shown in fig. 75).

Since no major differences in the mitochondria or the endoplasmic reti-

culum of the macula densa cells in these experiments were found when compared to control rats, the relatively decrease in hexosemonophosphate shunt enzymes activity may be due to the presence of less active light cells.

GENERAL DISCUSSION

One of the most reliable technique for staining the granules of the JG cells is that of Bowie (22). However, as it has been pointed out by several authors (596, 601) the method as originally described was time-consuming and presented several technical difficulties which rendered the results variable.

We have achieved satisfactory results by introducing modifications in several procedure steps involved in the technique and which have been extensively discussed in the appropriate chapter. This steps involve : a) fixatives modifications, b) shortening of the mordant period, and c) improvement of contrast by increasing the concentration of the neutral stain and by the use of another neutral stain (methyl violet mixture). Furthermore, since it is important to correlate light and electron microscopic examinations of the JG cells, we have been able to apply such procedures to glutaraldehyde-fixed kidneys with similar results.

Established histochemical methods for demonstrating the activity of various dehydrogenases were used. However, since they have various limitations which were also previously pointed out by their own authors (157, 296), several modifications were made. In the case of methods for detection of dehydrogenases of the hexosemonophosphate shunt $BaCl_2$ and $CoCl_2$ were used simultaneously in the incubating media containing Nitro-BT or Tetranitro-BT. In the case of the technique developed by Hess and Pearse (251) for the demonstration of α-glycerophosphate dehydrogenase, Nitro-BT

or Tetranitro-BT was used instead of MTT.

An index of the hexosemonophosphate shunt enzymes activity of the macula densa was devised in order to measure semi-quantitatively the activity of such enzymes.

The reproducibility of the techniques used and the reliability of the index were consistently verified by re-examining slides without previous knowledge of the experimental protocol. The macula densa index (MDI) has proved to be very useful when results of large series of animals must be compared especially in relation with the juxtaglomerular granulation index (JGI).

Our accidental observation that the complete ligature of the aorta between the two renal arteries in rats weighing over 300 g. results in atrophic left kidney accompanied by hypertension introduces a simple and reproducible method in the production of experimental hypertension. This procedure has not appeared in the literature as yet. However, personal communication have made it available to other workers in whose hands it appears to be equally effective and reliable (593, 594).

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Healthy rats of similar body weight but of different strains and sexes were studied for possible differences in JGI and MDI. No differences were observed, although some rats independently of the strain or sex seemed to have higher JGI and MDI, than others.

A further observation of probable but unknown functional significance was the finding in the epithelium of the renal pelvis of granular epithelial

cells, showing a high activity of the hexosemonophosphate shunt enzymes.

The distribution of the JGI and MDI in the renal cortex of the rat showed a correlation of these two parameters, both being higher in the outer cortex and lower in the corticomedullary region. However, when the lateral renal cortex (LRC) and medial renal cortex (MRC) were studied (see fig. 30 p.111) no correlation was found. JG cell granularity was higher in the LRC than in the MRC. Moreover, MDI was equal in both sides. Further work will be necessary to assess the possible functional significance of this observations.

The distribution of the JG cell granularity was studied in healthy dogs and beavers, possessing long and short Henle loops, respectively. Once more, in dogs, a difference was found in JGI between LRC and MRC. JGI was higher in the outer cortex in both dogs and beavers than in the corticomedullary zone. G6PD activity in dog macula densa was shown to be lower than in rats.

These observations emphasize the importance of counting (JGI, MDI) in the slides the total renal cortex in order to get a semi-quantitative evaluation of the changes occuring in the JG complex.

The difference in distribution of the JGI and renin content in the renal cortex seems difficult to explain when the superficial and deep part of the cortex are considered. We have presented evidence that it does not depend on the length of the Henle loops as postulated by Friedberg (48), since both dogs and beavers presented similar patterns of distribution.

The differences in distribution of the MDI from the superficial to deep parts of the cortex in normal rats and which are similar to those of the JGI, suggest that both parameters may be related to one another. Tubular sodium content and osmolarity in the region of the macula densa could be different between the superficial and deep parts of the cortex and both JGI and MDI could be influenced by them.

Differences in nerve supply of the efferent arterioles have been described in glomeruli of the outer cortex and the juxtamedullary cortex (362). Thus, in the former practically no nerve fibers have been detected while in the latter a highly rich supply has been observed. The opposite situation is encountered in terms of renin content, in the afferent arteriole of these glomeruli (very high content in the outer cortex and very poor in the juxtamedullary region). This discrepancy might bear a functional significance. One of the theories of the action of renin is its constriction of the efferent arteriole through the production of angiotensin (198). It would seem logical that this mechanism could be operative in the outer cortex. However, in the juxtamedullary cortex it would appear that the vasoconstriction might be largely under nervous control.

In considering the stretch receptor theory of Tobian (137, 203) another possibility could well be a pressure difference in the afferent arterioles of the superficial and deep part of the cortex.

Studies on renal hemodynamics indicate that the autoregulation of renal blood flow occurs primarily in the outer cortex and that the juxtamedullary zones are capable of only limited regulation (378). The presence

of relatively low JGI and MDI in this zone might be of interest in this respect.

In normal rats and dogs, granular epithelioid cells were also found in the efferent arteriole of the glomerulus. However, these findings are relatively uncommon. Granular cells in the afferent glomerular arteriole near the interlobular artery was a relatively common finding in rats.

Little is known about the degree of granularity of the JG cells in relation with renin secretion. The present series of experiments have provided direct evidence that, in some chronic experimental conditions affecting sodium balance, an increase as well as decrease of JG cell granularity can represent a state of renin hypersecretion. Moreover, the largest values of plasma renin activity were encountered when a marked degranulation occurs. (as in rats 2 weeks after adrenalectomy), this finding demonstrates that the JG cells can also follow a degranulation process in the same manner as other secretory cells when they are stimulated.

In our studies, no quantitative correlation was observed between the degree of JG cell granularity, plasma renin activity and G6PD activity of the macula densa of rats receiving various sodium intakes, or in adrenalectomized animals. This observation raises many questions concerning the role of the macula densa in renin production. The hexosamonophosphate (HMP) shunt enzymes have been implicated in multiple functions and one of them is the sodium balance. Changes in sodium balance must be very marked in order to give significant histochemical variations as in the case of adrenalectomized rats or DCA + NaCl treated animals.

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The electron microscopic observations showing the presence of secretory vesicles in the Golgi apparatus and, the continuity between the cisternal material of the endoplasmic reticulum and the granules matrix during their formation, indicate that the JG cell is following a similar morphologic pattern in the formation of secretory granules as other secretory cells. However, the release of the granule content seems to be different and the following hypothesis is proposed. The presence of granules incompletely surrounded by membranes and the presence inside such granules as well near the JG cell membrane, of microspheres of similar characteristics, suggest that such microspheres might well represent the form of release of the biologically active material (renin) in the JG cells.

The fine morphology of the macula densa showed that, in sodium- restricted rats, the Golgi apparatus became in some cells very prominent, and did not always keep its basal localization. This finding, in relation with the enlarged intercellular compartment, suggests that some re-absorptive processes are occuring in the macula densa cells. No discontinuity of the macula densa basement membrane was observed.

Although it has been suggested that the lacis cells can be transformed into JG cells, our findings do not support this hypothesis. The lacis cells did not contain any granule of the same type present in the JG cells and they did not present any secretory characteristics.

Fine morphological evidenee has been given of the transformation of the arteriolar smooth muscle cells into JG cells. A morphologically active process seems to occur during the stages of their transformation in which the morphological equipment of the smooth muscle cells is completely modified to a secretory cell. Granular epithelioid cells have been also encoun-

tered in the media of other renal arterioles and small renal arteries.

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The findings of a rich nerve supply of the rat JG cells in the afferent arteriole of the glomerulus give the morphological basis for the influence of the nervous system on the secretion of the JG cells, as one of the postulated mechanisms for renin secretion.

Furthermore, it is suggested that the vesicular processes described might well represent possible synaptic end nerving similar to those found at the myoneural junctions in skeletal muscle. The presence of granular vesicles in such vesicular processes also suggests that the innervation of the juxtaglomerular cells is adrenergic in nature.

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Tubular fluid content at the macula densa level has been emphasized as one of the stimuli which cause JG cell changes and renin secretion (see p.63). We have studied the "endocrine kidney" produced by our method (complete aorta ligature between the two renal arteries + ligature of the ureter) and by the technique described by Selye and Stone (505). This experimental model was chosen because no urine is formed and, therefore, no filtrate reaches the macula densa. Results showed that, at least in this experimental model, the JG cell granularity, plasma renin activity and the G6PD activity are independent of the urine formation. No relationship of any of these parameters with blood pressure was observed. Furthermore, in the experiments with "endocrine kidney", the G6PD activity of the

macula densa is unrelated to the degree of JG cell granularity, showing some degree of independence of the macula densa, in regard to the JG cells.

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The findings of light and dark granulated cells in the glomerular afferent arteriole of the "clipped" kidney of renal hypertensive rats as well as the observation of light and dark macula densa cells in the contralateral kidney raise the question of possible intracellular edema.

Although the Golgi apparatus in the macula densa cells is generally localized in the basal or parabasal region, it was observed that in some macula densa cells of the contralateral kidney of renal-clip-hypertensive rats, it was localized in the paraluminal region. These results might indicate a different functional state of some macula densa cells.

The morphological pattern of the macula densa in severe renal ischaemia is largely distorted, nevertheless, some clusters of cells were observed. In such cells, the only morphological feature in relation with the G6PD activity was a well-developed endoplasmic reticulum, the abundance of free ribosomes and the appearance of mitochondrial swelling, without any increase in their numbers. Therefore, it is difficult to explain the increased G6PD activity in such macula densa cells only by the localization of that enzyme in the mitochondrial.

The lipofuscin droplets in the macula densa might possibly be related to the increased G6PD activity, since the HMP shunt is closely related to lipid metabolism.

The present findings strongly suggest a morphological and functional independence of the macula densa cells from JG cells for renin formation and secretion. However, it does not rule out the possibility that changes of the interstitial tonicity around the macula densa or other sections of the nephron more or less in contact with the JG cells could influence the activity of the JG cells. Other factors such as the nervous system, blood volume or perfusion pressure must be considered in order to explain the changes in JG granularity and renin secretion. It appears that the stimuli acting on the JG cells may be the summation of a complex and variable interaction among all these factors.

CONCLUSIONS

Studies of the juxtaglomerular complex under normal and experimental conditions led to the following conclusions :

1. Helly's modified fluid or Stieve's modified fluid were the best fixatives for demonstrating the JG complex with the Bowie modified method.

2. The combined addition of $BaCl_2$ and $CoCl_2$ in the incubating medium for histochemical demonstration of hexosemonophosphate shunt enzymes in the presence of Nitro-BT or Tetranitro-BT, gives excellent histochemical results. These techniques are highly reproducible.

3. The semi-quantitative macula densa index (MDI) of G6PD activity devised by the author appears to be a reliable and useful procedure when a semi-quantitative analysis is indicated. This MDI permits to make statistical evaluations and compare the results with other parameters.

4. The simplicity of the method described to produce atrophic kidneys accompanied by hypertension is highly reproducible. This experimental condition is often accompanied by cardiopathy.

5. No significant differences in JGI and MDI were observed in rats in relation to their sex or strain. However, a zonal distribution of the JGI and MDI in the renal cortex was observed, being higher in the outer cortex than in the corticomedullary region. The same zonal distribution of the JGI was also pointed out in dogs and beavers.

6. An increase of the JGI accompanied by an increase of PRA was observed in adult rats receiving a sodium-deficient diet. However, no significant changes occur in MDI. High sodium intake in rats produced a marked decrease

of JGI, without significant decrease in the MDI, or in PRA. Moreover, when DCA is added, a significant decrease of the three parameters was observed.

7. Adrenalectomized rats can either display a state of hypergranularity or degranulation of the JG cells depending on the sodium intake, in either case PRA and MDI are increased. However, the higher PRA values were observed in those rats with the most marked degranulation of JG cells.

8. The fine morphological appearance of the JG cells in normal rats and in rats under various experimental conditions (sodium restriction, renal ischaemia, and mercuric bichloride treated rats) strongly suggest that the formation of JG granules follows a similar pattern as other secretory cells. The intensive study of the fine morphology of the "lacis cells" does not support the concept of transformation of such cells into JG cells.

9. Under experimental situations which increase the JGI evidence was obtained to demonstrate the origin of the JG cells from arteriolar smooth muscle cells.

10. The fine morphologic study on the innervation of the JG cells has shown that vesicular nerve processes containing granular vesicles are often associated with JG cells. Intervals between 700 to 2.000 A^{o} were found between the axolemma of the vesiculated processes and the JG cell membrane. The intermembranous space is filled by the basement membrane of the arteriole.

11. The fine morphologic study of the macula densa shows the following findings :

a. No discontinuity of the basement membrane of the macula densa was observed in any experimental studies.

b. In sodium restricted rats, marked sub-basilar compartments were observed, as well as a prominent Golgi apparatus which in some cells loses its usual basal localization.

c. The cluster of macula densa cells observed in the clipped kidney of renal hypertensive rats presented also marked sub-basilar compartments mitochondrial swelling without an increase in number, increased endoplasmic reticulum and lipofuscin-like droplets.

d. The macula densa of the kidney contralateral to the clipped one has shown light and dark cells. In some dark cells, the Golgi apparatus is localized paraluminally

12. In "endocrine kidneys" the JGI, PRA and in many instances the MDI were increased in spite of the fact that no urine is formed and no tubular fluid reaches the macula densa.

In summary, it appears that :

a) A state of hypergranulation as well as degranulation of the JG cells can be followed by an increase in plasma renin activity.

b) A morphological independence can be found between the components of the juxtaglomerular complex.

c) Both JG cells and the macula densa may be involved in the renal regulation of sodium balance.

d) JG cells have a rich nerve supply, probably adrenergic in nature.

e) Tubular sodium content at the macula densa level does not appear to play a major role in JG cell granularity and renin release in our experiments.

CLAIMS TO ORIGINALITY.

The studies contained in this thesis contribute several original observations.

From the point of view of methodology, advantageous modifications have been introduced to histological (fixatives and stains) and histochemical (HMP) procedures, rendering them more reproducible and less-time-consuming. The author has devised a method for the semi-quantitative determination of G6PD activity in the macula densa (MDI). The author also proposes the ligature of the aorta between the two renal arteries as a simple and reproducible new technique for the production of experimental hypertension in rats.

A new approach to the study of the juxtaglomerular complex has been presented which consists on the simultaneous exploration of a triad of parameters. This approach has enabled the author to examine the juxtaglomerular cell granularity (JGI), G6PD activity of the macula densa (MDI) and the plasma renin activity (PRA) in the same experimental preparation using intact, or adrenalectomized animals as well as rats with an "endocrine kidney". The major findings of this correlative study under various conditions of sodium intake, are summarized in "Conclusions" and need not to be repeated here.

A comparative study of the entire renal cortex in species with different types of nephrons has also been undertaken for the first time. The results indicate that there is no relationship between JG cells granularity and the length of Henle's loop.

Further contributions have been derived from the studies with the electron microscope. Evidence is presented for the origin of JG cells from arteriolar smooth muscle cells. New aspects concerning the innervation of JG cells have also been explored. Macula densa cells have also been studied in sodium-restricted rats and renal-clip-hypertensive rats.

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