Laboratory Diagnosis of Kidney Diseases

edited by

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LABORATORY DIAGNOSIS OF KIDNEY DISEASES

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Preface

This book contains the edited proceedings of an Applied Seminar on the Laboratory Diagnosis of Kidney Diseases, held in Washington, D.C., under the auspices of the Association of Clinical Scientists. In organization and format, this volume is similar to the published proceedings of eight previous seminars.

Never in the history of civilization has scientific knowledge advanced with such rapidity as it does now. Compared to the last century, the advancements during this century represent a great upheaval. In recent years, one of the most striking changes in clinical science is the accelerated rate of change itself. The Applied Seminars of the Association of Clinical Scientists are designed to cope with these changing times so that the dissemination of knowledge in clinical science may keep pace with the acquisitions.

The furtherance of clinical science depends in large measure upon developments in methodology. It is for this reason that a number of procedures are included in this volume which are not currently undertaken in many clinical laboratories; nevertheless, in our opinion these procedures may play increasingly important diagnostic roles in future years. It is our hope that clinical scientists will find the chapters on methodology helpful in initiating newer procedures in their laboratories. It is also our hope that the chapters pertaining to fundamental considerations and clinical interpretations may be useful in applying the current concepts of renal diseases to patients at the bedside.

Our grateful appreciation is expressed to the lecturers who have generously contributed their time and energies to the success of the Applied Seminar and to the preparation of these proceedings. Our thanks are given to our publisher, Mr. Warren H. Green, and his staff for their gracious cooperation.

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Chapter 1

Anatomy and Ultrastructure of the Kidney

JENO E. SZAKACS, M.D.

INTRODUCTION

The kidneys are vital excretory organs that regulate the internal environment of the body. In adult man, they weigh 120 to 150 gm each and through their vascular tree flows approximately 1200 ml of blood per minute, some 25% of the total cardiac output. The vascular and epithelial elements form special functional units, the nephrons, consisting each of a *glomerulus* and a *tubule.*

The special apposition of blood vessels and tubules gives a characteristic gross appearance to the kidney and this organization assures the required degree of function. A renal cortex and medulla can be differentiated on a cut surface of the mammalian kidney, and the demarcation is usually enhanced by the arcuate arteries, though not in all species. The cortex contains all glomeruli and the convoluted tubules. The medulla contains some or all of the parallel descending and ascending segments of the renal tubules, called the loops of Henle, and the collecting ducts. The schematic diagram (Fig. 1) from Pitts (13) illustrates the organization of the different layers of the kidney. In man, most nephrons are cortical and only 1/8 of the nephrons are juxtamedullary, with long thin loops of Henle extending into the renal pyramids, while in desert animals most or all nephrons are provided with long thin loops of Henle that allow them to conserve water by concentrating the urine to a high degree. Pitts' diagram also illustrates the relationship of blood

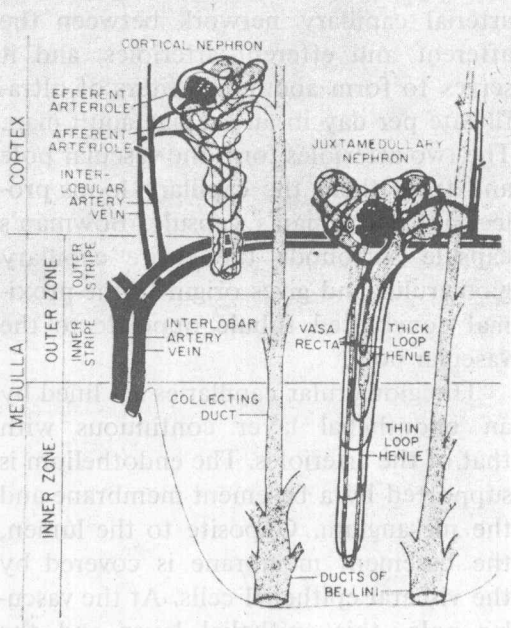

Fig. 1. Distribution of nephrons in the different layers of the kidney. Drawing from Pitts (13).

vessels to the nephron. Blood is carried through interlobar branches of the renal artery to the arcuates, interlobular arteries and afferent arterioles to the renal glomerulus. It leaves the glomerulus via the efferent artery which provides branches to form vascular loops around the convoluted tubules, and the vasa recta to parallel the loops of Henle. The venous blood is emptied into the interlobular veins and returns to the renal veins by vessels paralleling the similarly named arteries.

The following is a brief illustrated description of the salient points and of the more recent information about renal structures, including that obtained by electron microscopy. Much has been written on this subject. Classical histologic techniques culminated in the works of Möllendorff (20) and Goormaghtigh (4) in the 1930's. More recently, classical chapters on the ultrastructure of the kidney were written by Rhodin (16), Spargo (18), and Latta (10).

THE RENAL GLOMERULUS

The structure of the renal glomerulus has been studied extensively and our understanding of its function is better than that of the tubules. The identification of the anatomical site where each of the physiological processes takes place is the great challenge of our time.

The renal glomerulus is a specialized arterial capillary network between the afferent and efferent arterioles, and it serves to form about 180 liters of ultrafiltrate per day in an average adult man. The two arterioles form the vascular pole and from there the capillary loops project into Bowman's capsule. Bowman's capsule surrounds the entire capillary glomerulus and gives origin to the proximal convoluted tubule opposite to the vascular pole.

The glomerular capillaries are lined by an endothelial layer continuous with that of the arterioles. The endothelium is supported by a basement membrane and the mesangium. Opposite to the lumen, the basement membrane is covered by the visceral epithelial cells. At the vascular pole, this epithelial layer and the basement membrane reflects to form Bowman's capsule. The epithelium becomes flattened and is referred to as the parietal epithelium of the glomerulus (Fig. 2).

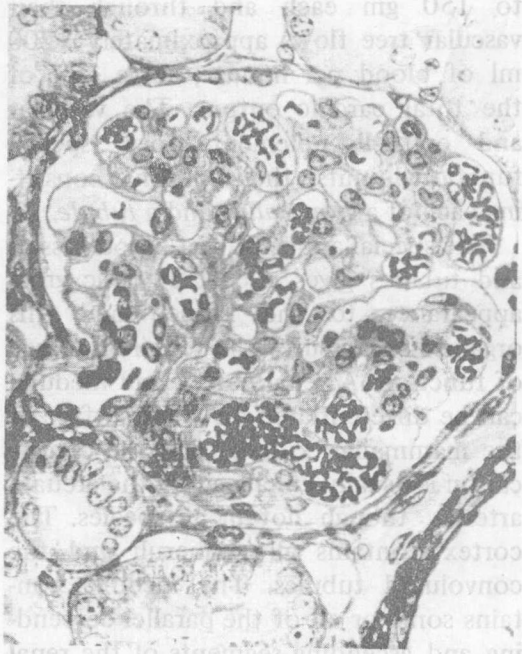

Fig. 2. Photomicrograph of the renal glomerulus. Note the vascular pole, macula densa and lacis. The basement membrane at the macula densa is ill defined and continuous with the fibrils of the lacis. Only the afferent artery is in the plane of section. The capillary basement membrane, endothelial and epithelial cells are easily identified at the periphery of the lobules. (x 500)

Silver impregnation and thin sectioning permitted the light microscopist to visualize the capillary basement membrane with its reflections and continuity with that of Bowman's capsule, but only electron microscopy allowed resolution of the fine structure of the mesangium between the capillaries. The mesangial space between two or three capillary loops is filled by cells and intercellular matrix and it is enclosed by the endothelial side of the basement membrane. The mesangium provides points of attachments for the basement membrane (Bohle and Herfarth (1)).

The Capillary Endothelium. The capillary wall is supported by the basement membrane at the periphery and by the mesangial cells between capillary loops. Endothelial cells line the entire capillary lumen covering the basement membrane and at the mesangium they are directly apposed to the mesangial cells. The nucleus is usually in the mesangial area and is surrounded by cytoplasm containing most of the cellular organelles: The Golgi apparatus, mitochondria, some rough endoplasmic reticulum and the centriole. The remaining cytoplasm is attenuated, forming a thin layer covering the peripheral portion of the capillary. On cross-section the attenuated endothelium appears discontinuous with interruptions at regular intervals. Tangential sections reveal round or elliptical openings about 1000 Å in diameter. The apparent openings are referred to as endothelial "fenestrae." High resolution electron micrographs reveal the fenestrae to be closed by a thin diaphragm (Rhodin, 1962 (14)). According to Ito (1964) (8), this diaphragm is formed by two outer protein layers of the plasma membrane, while the inner protein and lipid layers reach only to the edge of the fenestration. This protein-mucoprotein diaphragm is considered by Rhodin to provide the kidney's filtering action,—thus permitting small molecules to pass through while retaining larger ones. In addition, large molecules were shown to be actively transported through the endothelium (Meneffe (11)), from the lumen both to the basement membrane and to the mesangium or juxtacollicular region in a form of modified phagocytosis. Pinocytotic vacuoles are seen as well as a complex system of vacuoles in the endothelial cytoplasm close to the mesangium. These participate in the transport of fluids and several other particles including those experimentally marked, such as globin.

Glomerular Basement Membrane. The basement membrane envelopes the capillary loops together with the mesangium much the same way as the mesentery envelopes the intestinal tract. The reflections of the basement membrane are well illustrated in Figures 2, 3 and 4. The illustrations also show that in preparations stained with uranyl acetate and lead hydroxide the basement membrane is resolved into three layers—a central dense layer and an inner and outer electron lucent layers. The thickness of the basement membrane varies from species to species. In man, it measures 3500 Å. Electron micrographs of fixed material reveal a fibrillar substructure that impressed many investigators by its similarity to filters. It was thought that the basement membrane could form a filtration barrier, but more recent evidence indicates that the basement membrane is a thixotropic gel. This would explain diffusion of small molecules through the basement membrane and retention of some larger ones while large aggregates would produce enough mo-