Electron Microscopy of the Kidney

In Renal Disease and Hypertension

A Clinicopathological Approach

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To my infinitely patient wife, Pranati, and
my two beloved daughters, Aditi and Atashi,
who are the source of my persistence and perseverance

This book is a product of the studies in the
Renal Electron Microscopy Laboratory, which has been
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Foreword

In one golden age of medicine epitomized by William Osler, the physician also aspired to mastery of gross and microscopic pathologic anatomy. Now another such age has dawned in which ultrastructure and immunopathology provide insights into mysterious diseases of the kidney, connective tissues, joints, and muscles, among other sites.

Dr. Anil K. Mandal has a background in clinical nephrology, experimental pathology, and diagnostic pathology of renal diseases that suits him well for his chosen task. This is to explain clearly the clinicopathologic entities seen by nephrologists, using the full range of available morphologic techniques. His approach is brisk and incisive. To read his monograph as a pathologist is to make oneself a better clinician, and as a physician is to improve one's grasp of pathology. Such correlative knowledge seems at present the means most likely to lead to the ultimate control of some crippling chronic renal diseases.

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Preface

The idea of writing a book on "electron microscopy of the kidney in renal disease and hypertension" has emerged from frequent requests by medical students and physicians in training for a comprehensive monograph on electron microscopy of normal and diseased kidneys. The current textbooks dealing with the pathology and clinical diseases of the kidney describe the fine structure of the normal kidney but few, if any, have elaborated the fine structure of the diseased kidney. Electron microscopy studies of renal tissue in humans and animals have advanced our knowledge significantly and allowed a better understanding of the pathology, pathogenesis, activity, and progression of acquired renal diseases. However, the literature concerning these studies is scattered so that the collection and retrieval of these data involve considerable time and effort from students and practicing physicians. Thus, the inquisitiveness of the potential readers, accompanied by the lack of a compact monograph on the fine structure of the diseased kidney, has encouraged me to write this book.

During the past 17 years, my research concerning the histopathology of the kidney in renal disease and essential hypertension in humans and experimental animals has facilitated my understanding of renal fine structure in health and disease. The additional clinical responsibility of studying and reporting electron microscopy of renal biopsies from several hundred patients during the past 7 years has also added confidence to my capabilities. My decision to undertake the difficult task of writing this book has been inspired by Dr. Emanuel Rubin, who has raised the morale of clinicians with the following comments: "The clinician who restricts himself to a narrow subspecialty is in a position to become expert not only in the clinical, physiological, and biochemical aspects of this area, but also in the morphological expression of the disease" (Editorial. *Human Pathology* **6:**127, 1975). In addition, the generous encouragement of Dr. Sheldon C. Sommers has been of paramount importance in my successful completion of this piece of work.

This book, featuring electron microscopy studies, is based on a correlation with light microscopy pathology. The goal of this book is to provide medical students and physicians engaged in practice or research with a comprehensive but discrete reference on the fine structure of the kidney in renal disease and essential hypertension. Attempts have been made to avoid any confusion caused by a variety of nomenclatures and classifications. It is my utmost desire that this book be relatively unburdened by literature references, especially those that are primarily concerned with conflicting and contentious reports. To fulfill the objective of a free-flowing textbook, I have not interrupted chapter texts with reference numbers. At the end of each chapter, a list of pertinent references in alphabetical order is included in order to facilitate a detailed review by the reader.

This book is highlighted by discussions of the newer concepts of classification of glomerulonephritis, the pathogenesis of renal lesions in essential hypertension, and the antihypertensive role of the renal medulla and papilla. The fine pathology of the nephron is related to patient profiles by several illustrative clinical reports. Adjunctive tests are suggested to support interpretations of the anatomic pathology of the kidney and to establish the clinicopathological syndrome. Each chapter concludes with a brief summary for a quick grasp of its entire contents.

Electron microscopic features suggestive of activity and progression of the pathological process are indicated. The disparities between light and electron microscopy in the study of renal diseases and essential hypertension are elaborated. It is important from the viewpoint of economics to know the necessity of the routine use of electron microscopy in the clinical practice of nephrology (renal disease). The concluding chapter outlines the principles of management pertaining to the principal changes in the kidney in different types of renal diseases.

Finally, it is my sincere belief that this monograph should prove useful to clinicians and pathologists for the purpose of a rapid review of renal diseases and hypertension, as well as to medical students and house physicians preparing for specialty board examinations.

A.K.M.

Acknowledgments

I would like to take this opportunity to express my deep gratitude to Dr. Sheldon C. Sommers of Lenox Hill Hospital, New York, for his continuous encouragement in the initiation and completion of this book. Without my association with the benevolent Dr. Sommers, it would not have been possible for me to accomplish this task. The time, effort, and contributions of Dr. James E. Wenzl, Professor and Vice-Chairman, Department of Pediatrics, Children's Memorial Hospital, and the University of Oklahoma College of Medicine, Oklahoma City, Oklahoma, are deeply appreciated. I am also grateful to Dr. Jack Metcoff, George Lynn Cross Professor of Medicine at the University of Oklahoma College of Medicine, for his encouragement to write this book and for his ideas about its style. I am deeply appreciative to Mrs. Carolyn Clay and Mrs. Reta Weaver, Renal Section secretaries, for their kind efforts in completing this book. I am pleased to express by gratitude to two other secretaries, Mrs. Alanda Anglin and Mrs. Billie Acree, of Pediatric Nephrology, Children's Memorial Hospital, Oklahoma City, Oklahoma, for their kindness and desire to complete this book. I am thankful to Dr. Katerina Chrysant, who has been my research fellow; Joseph Uzupik and James L. Borke of the Electron Microscopy Laboratory, West Surburban Hospital, Oak Park, Illinois; Sandi Shustak, Electron Microscopy Laboratory, Lenox Hill Hospital, New York; Mrs. Alta Alkire, Mrs. Valenda Teeter, and George O'Shea of Medical Media Production, Veterans Administration Hospital, Oklahoma City; Linda Jackson, Medical Photography, Children's Memorial Hospital, Oklahoma City; and Lynette Southern of the Medical Library, Veterans Administration Hospital, Oklahoma City, for their assistance. I am thankful to Dr. Robert D. Lindeman, Veterans Administration Hospital, Louisville, Kentucky (formerly Chief, Renal Section, VA Hospital and University of Oklahoma College of Medicine) and Dr. Anthony W. Czerwinski, Acting Chief, Renal Section, VA Hospital and University of Oklahoma College of Medicine, for sparing a part of my time from Section activities, which facilitated

completion of this book. Finally, I am grateful to Mr. Carlos Taylor of Chandler, Oklahoma, for financial assistance toward secretarial and photographic needs.

Overall electron microscopy assistance, particularly with Part 2 of Chapter 2, was provided by John A. Nordquist, B.A., B.S., M.A., M.S., Electron Microscopist, Renal Electron Microscopy Laboratory, Veterans Administration Hospital, Oklahoma City, Oklahoma.

A part of the electron microscopy assistance was provided by Marguerite Pata, electron microscopy technician.

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1 Microscopy for the Practicing Physician

HISTORY OF MICROSCOPY*

Microscopy dates from the seventeenth century when Robert Hooke and Marcello Malpighi used simple lenses in the study of various structural features. Between 1673 and 1716, Leeuwenhoek developed compound lenses and published a series of observations upon protozoa, bacteria, muscle, nerve, and many other structures. By the early nineteenth century, the compound microscope had become highly developed. Robert Brown in 1830 discovered the nucleus; Schleiden in 1838 and Schwann in 1839 enunciated the "cell theory." In 1841, Henle published the first comprehensive account of human histology. Virchow described the human body as a "cell state" and listed specialized categories of cells in 1863.

TYPES OF MICROSCOPY*

Several types of microscopes are available for the study of biological material. Basically, they can be classified according to the type of light source used, and include the light (or optical) microscope, polarizing microscope, phase contrast microscope, interference microscope, dark-field microscope, x-ray microscope, electron microscope, and ultraviolet microscope. Of these, the light microscope is the one most commonly used in the study of biological specimens. Other microscopes frequently used for this purpose are the electron microscope and the ultraviolet microscope.

* The material presented throughout this section is from Chapter 1 of Leeson and Leeson (1970); used through the kind permission of Dr. Thomas S. Leeson, University of Alberta, Canada.

Light Microscope

The light microscope (LM) has three magnifying devices: (1) An objective lens provides the initial magnification; (2) an ocular lens magnifies the primary image; and (3) a condenser lens beneath the stage of the microscope concentrates the light from its source into a very bright beam. All three lenses provide sufficient light for a gross study of the magnified object. A brief description of the polarizing microscope is deemed necessary because of its application in the clinical practice of nephrology.

Polarizing Microscope

This microscope was developed by mineralogists in order to study crystalline materials. Many natural objects such as crystals and fibers exhibit double refraction or birefringence. In histological material, birefringence is caused by the orientation of particles too small to be resolved even by the best lenses. The polarizing microscope is a conventional light microscope in which a Nicol prism (or Polaroid sheet) is interposed in the light path below the condenser lens. This "polarizer" converts all light passing through the instrument into plane-polarized light or light which vibrates in only one optical plane. A similar second prism called the "analyzer" is placed within the barrel of the microscope above the objective lens. When the analyzer is oriented so that its polarizing direction is parallel to that of the polarizer below, one sees the regular image. However, if the analyzer is rotated until its axis is perpendicular to that of the polarizer, no light can pass through the ocular lens and the field is dark. A birefringent object, e.g., calcium or uric acid crystals (especially the latter), will become visible upon a dark background. In addition to these crystals, other biological materials, e.g., muscle fiber, connective tissue fibers, lipid droplets, exhibit birefringence.

Ultraviolet Microscope

Since ordinary optical lenses are nearly opaque to ultraviolet light, quartz lenses are used throughout the lens system. In general, this system allows an improvement in resolution about twice that of the ordinary microscope (0.1 μm). Ultraviolet light is also employed in the fluorescence microscope (FM). The specimen can be observed by its emitted fluorescence when ultraviolet light is focused upon it. The fluorescence is the result of pretreatment with fluorescein dyes.

ELECTRON MICROSCOPY

The electron microscope (EM) is an instrument designed and developed by physicists and engineers. It has a most complex intrinsic mechanism. The operation of this instrument is difficult at the outset, but becomes easier with experience.

Table 1-1
Milestones in the Development and Perfection of the Electron Microscope

Year	Scientists	Instrument
1932	Knoll and Ruska	1. Electron source 2. Two magnifying lenses 3. Resolution less than LM
1934	Ruska	Condenser lens was added
1935	Driest and Muller	Resolution greater than LM was obtained
1938	Von Borries and Ruska	Advancement in design resolution to as small as 100 Å
1939	Burton, Hillier, and Prebus	First operational electron microscope plant at University of Toronto, Canada
1941	Hillier and Vance	First commercially produced electron microscope in North America (capable of resolution as low as 25 Å)

Historical Background

The fundamental physical concept of electron microscopy dates to the latter part of the nineteenth century. The following theories led to the development and successful application of this instrument: (1) A moving electron can be assigned a very short wavelength (deBroglie, 1924). (2) A suitable magnetic or electrostatic field can be used as true lenses for an electron beam which will be capable of producing a reliable and even an enlarged image (Bush, 1926).

Milestones in the development and perfection of the electron microscope are shown in Table 1-1, from which it can be seen that an electron microscope was first used successfully in the study of biological specimens (tubercle bacilli) at the University of Toronto, Canada, in 1939. During the last three decades, continuous modification of the optical system has led to achieving resolution as low as 2 Å, more image intensification, and better photography.

Objectives of the Electron Microscope

The two objectives of the electron microscope are resolution and enlargement of the image. The resolution is defined as the minimum separation that produces a detectable change in contrast in an area of an image of two points and between the two points and is expressed in terms of angstroms (Å). The units in common usage are 1 mm (millimeter)= 1000 μm; 1 μm (micrometer)= 10,000 Å; and 1 mμ (millimicron) = 10 Å.

Objective Systems for the Operation of the Electron Microscope*

There are three major integrated systems required for the successful operation of the electron microscope: the illuminating, imaging, and image translating systems.

* The material presented in this subsection is from Wischnitzer (1962), used through the kind permission of the author and publisher.

The illuminating system serves to produce the required radiation and direct it onto the specimen. This system consists of the source, the emission of radiation from which an image can be formed, and the condenser lens assembly which regulates the intensity and the convergence of the illuminating beam on the specimen.

The imaging system consists of the lenses which produce the final magnified image of the specimen. The objective lens focuses the beam which passes through the specimen in order to form a magnified intermediate image. The projection lens (or ocular lens) magnifies a portion of the intermediate image to form the final image.

The image translating system converts the radiation into a visual image via a fluorescent observation screen that consists of a plate and fluorescent material. The fluorescent material, which is coated on the plate with gum arabic or collodion, is a mixture of finely divided particles of zinc and cadmium sulfides. The fluorescent screen provides the opportunity for (1) orientation and location of the desired field of view, (2) study of the general characteristics of the image of the specimen, (3) accurate focusing of the lenses, and (4) alignment of the entire instrument.

Principles of Electron Microscopy

The marked differences that exist between light and electron microscopy are shown in Table 1-2 and Fig. 1-1.

Photography is an essential part of electron microscopy. In my own opinion, study of the fine structures in the fluorescent screen should be followed by photography of the obscure and abnormal findings on 2 × 2 in. or 35mm films or

Table 1-2
Fundamental Differences in the Operative Mechanisms of Light and Electron Microscopes

Light microscope	Electron microscope
Fixed focal length	No fixed focal length
Necessary to change objective lenses for different magnification	Not necessary to change objective lenses at different magnifications; "magnification of the objective is fixed"
Source of illumination is natural or artificial light	Source of illumination is a beam of high-velocity electrons accelerated under vacuum
Different focal levels of the specimen can be seen relatively independently	Limited to a small area of the whole specimen
Image formation is due to differential absorption of light	Loss of electrons, scattering of electrons by individual parts of the specimen (image contrast)
Viewing directly into the specimen	Viewing the image of the specimen
Photography is optional to the operation	Photography is a requisite part of the entire operation

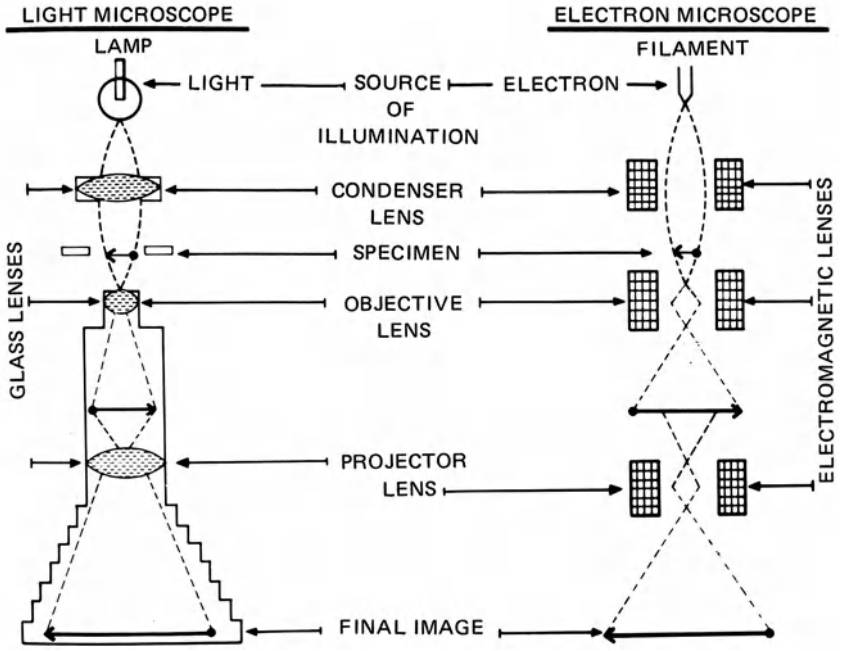

Fig. 1-1. Differences between the mechanisms of light microscopy and electron microscopy. Reproduced from Leeson and Leeson (1970) by the kind permission of the author.

2 × 2 in. glass plates. Enlargements and prints made from the negatives should then be studied thoroughly for proper interpretations of the findings and for establishment of the diagnosis.

Basic Needs for the Successful Use of the Electron Microscope

1. A suitable fixative to preserve the natural state of the specimen.
2. An embedding material to replace the volume originally occupied by the water in the specimen.
3. A sharp and durable cutting edge to secure thin sections.
4. An ultramicrotome with precision and reliability to cut sections thinner than 500 Å.

Fixative

The comparative study of a number of aldehydes by Sabatini, Bensch, and Barrnett in early 1960 (for details see Hayat, 1970; Kay, 1965) indicated that glutaraldehyde and acrylic aldehyde were the most effective morphological fixatives. Acrylic aldehyde was never used because of intolerable lacrimation evoked by this substance, so that since the mid-1960s, glutaraldehyde has been the most popular and widely used fixative in the field of electron microscopy. The quality of preservation of tissue is excellent when the tissue is fixed in

glutaraldehyde and postfixed in osmium tetroxide. A 4% glutaraldehyde solution is usually used; it is prepared by mixing a 25% stock solution of glutaraldehyde in distilled water with a phosphate buffer to provide a 4% solution of glutaraldehyde in 0.1 M buffer at pH 7.4. The solution can be stored in a bottle in the refrigerator for weeks. However, in case of infrequent use, the solution should be checked periodically for clouding, precipitation, and change in the pH. In case of slight precipitation at the bottom of the solution, the supernatant portion remains suitable for use, but if the precipitate is found throughout, the entire solution should be discarded.

A great advantage of the use of glutaraldehyde as the primary fixative is that it allows preservation of the tissue for a long time. To ensure stable preservation, the tissue should be removed from glutaraldehyde and stored in 0.1 M phosphate buffer at pH 7.4. In this way the tissue can be stored for weeks or months. Just before dehydration, the tissue should be postfixed in osmium tetroxide. *Note of caution:* After initial fixation, postfixation, or long-term fixation, the tissue must be stored in a *refrigerator* and *not in a deep-freeze*.

Embedding

Epon and Spurr are the two types of embedding mixtures commonly employed. Epon 812 (sold as Epikote in Europe) is the most widely used embedding resin for electron microscopy. This resin, because of its low viscosity, penetrates into the tissues faster than Araldite. The distinct advantage of Epon over other embedding materials is its rapidity, so that the whole process of washing, dehydration, infiltration, and embedding is completed in less than 3 hr. No difference was found in the appearance of the fine structure between the tissue processed by this rapid method and that processed by the standard conventional method. This rapid method should prove useful as a quick diagnostic aid in clinical practice, especially if the results of light microscopy study are obscure. Spurr introduced ERL 4206 as an embedding medium in electron microscopy. ERL 4206 has the lowest viscosity of all the known plastics used in electron microscopy. The very low viscosity facilitates its rapid penetration into the tissues.

In my own experience, the quality of thin sections does not seem to be better with one type of embedding than with the other. However, the opinions of the technicians differ, some seeming to prefer one over the other from the standpoint of thin sectioning. For more details concerning fixatives and embedding materials, see the book by Hayat (1970).

Successive Steps in Electron Microscopy Study

The successive steps involved in the completion of EM study are shown in Fig. 1-2. It should be remembered that if mistakes are made in any of the steps, the final analysis will be adversely affected. In well-prepared sections and with careful staining by the use of freshly prepared reagents, artifacts are generally

slight or none. On the contrary, sloppy work, a bad knife, lack of confidence, and miscalculation will cause total failure of the study. The failure is attributable to poor penetration of the electron beam by the inadequately prepared specimen, the result being an inadequately visible light image. In addition, a variety of artifacts will greatly interfere with the understanding of the changes and interpretation of the photographic findings. The time required for the completion of the study varies from 48 to 72 hr. This time factor is extremely important from an economic point of view since preparation ties up highly paid technical personnel. Thus, failure of such study simply means a financial loss.

Optimum States for Electron Microscopy

The optimum conditions for ideal electron microscopy study are living tissue and fixation of the tissue within seconds of removal from the living organism. The general consensus of opinion is opposed to the use of autopsy material for electron microscopy, although attempts have been made to utilize autopsy tissue for such study. I have studied postmortem kidneys from hypertensive cases. In general, the basement membranes of the glomeruli, tubules, and arterial vessels

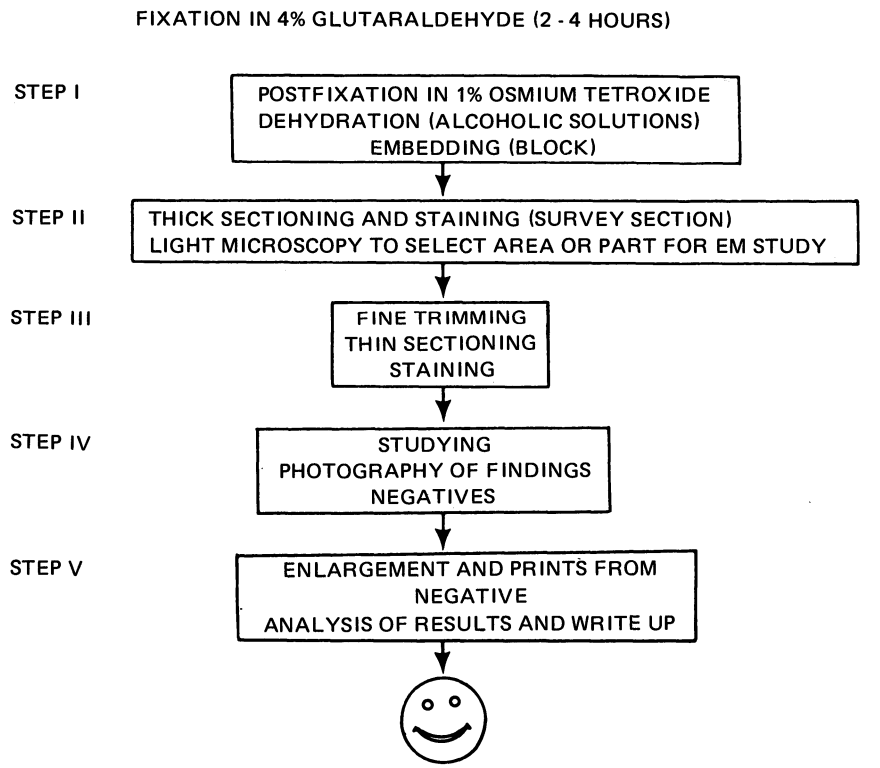

Fig. 1-2. Steps of electron microscopy study.

remain reasonably intact. The glomeruli and the arterial vessels do not demonstrate discernible autolytic changes until 24 hr after death, but the tubular cells undergo swelling and vacuolization, and profound edema appears in the interstitium. In the latter components, any attempt at interpretation of findings beyond autolytic change is futile. Therefore, in my own experience, electron microscopy of postmortem material does not serve any fruitful purpose other than to satisfy curiosity, and cannot be justified.

The Purpose of Electron Microscopy in Biological Studies

The most important goal of this technique is to resolve cellular constituents, membranes, and other structures. The cellular constituents which cannot be resolved by light microscopy include mitochondria, Golgi complexes, endoplasmic reticulum, nuclear contents, ribosomes, membranes (e.g., cellular membrane, nuclear membrane, plasma membrane infoldings), secretory granules, glomerular foot processes, and other structures (e.g., viral particles, inclusion bodies).

Electron Microscopy in Clinical Practice

Electron microscopy has improved our knowledge of the fine structures of many organs in the mammalian body. The greatest contribution of this technique is to studies of the anatomy and pathology of the kidney. In fact, the earliest reported EM study of the organs was that of the kidney by Pease and Baker in 1950. With sophistication of the technical aspects, knowledge concerning the fine structures of the kidney has continued to grow. When the features of the fine structures of the kidney were grasped, investigators began to study kidney tissue obtained through percutaneous biopsies from patients with glomerular diseases. One such study was first reported in the English literature by Farquhar *et al.* (1957). (For details of the aid of electron microscopy in the practice of nephrology, see Chapter 12.)

Ethics in Electron Microscopy Study

The EM laboratory is generally located in the basement of a hospital or institution. This type of location has the advantage of avoiding vibrations which occur most commonly in the upper floors of the building and which cause interference in the operation of the machine. But this odd location, coupled with lengthy processes and the need to study in complete darkness, causes a great deal of frustration and tends to distract both technical and professional personnel. Although the unique value of electron microscopy may lure many sophisticated individuals, its tedious practice discourages their interest.

The professional responsible for the EM laboratory for medical sciences may be an M.D. or a Ph.D. investigator. The M.D. is most commonly a pathologist, but infrequently the individual is an internist or other specialist. Customarily, the

time spent in the EM laboratory by the professionals is less than that spent in other types of microscopy or laboratory work. This is caused in part by a lack of adequate training and interest, and is detrimental to the ethics of EM study.

In my own judgment, the investigator should spend ample time, if possible, in studying the sections, whether they are from patients or from animal research material. He/she should identify the changes, photograph the important and obscure findings, and make notes of his/her study. The findings should be verified in the prints, along with missed observations, before a final diagnosis is made.

Therefore, the ethics of EM study states that both technical and professional personnel must be well trained, persevere, and develop adaptability in order to work under adverse circumstances. Without these characteristics, personnel may fail to achieve their goals.

The costs of hiring personnel and purchasing essential supplies are so high that serious consideration must be given to the aforementioned factors before designing an EM laboratory.

SUMMARY

1. Among the eight different types of microscopes listed, the light microscope, electron microscope, and ultraviolet or fluorescence microscope are the most commonly used microscopes for the study of biological specimens.

2. The technical aspects of LM and FM are simple as opposed to the complex mechanisms involved in the operation of EM.

3. The intricate operation of EM coupled with the excessive time involved in the preparation and study of specimens decreases the interest of many professionals.

4. It must be remembered that immediate fixation of the tissue in a suitable fixative (cold 4% glutaraldehyde) is an essential requirement for expected results.

5. Epon 812 or Spurr embedding media (ERL 4206) are the two most commonly used embedding media.

6. In EM study of biological materials, living tissue is an optimum need. The EM study of postmortem material does not serve any fruitful purpose and appears to be unjustified.

7. Perserverance, adaptability, and confidence are the keys to achievements in the field of EM.

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Renal Biopsy 2

PART 1
INDICATIONS, CONTRAINDICATIONS, TECHNIQUES, AND COMPLICATIONS • JUSTIFICATION FOR STUDIES OF LIVE RENAL TISSUES • ETHICS AND INDICATIONS FOR SERIAL RENAL BIOPSIES

INTRODUCTION

The development of a percutaneous technique to procure a small piece of tissue from one of the two kidneys has become a cornerstone in the study of renal parenchymal disease. Studies of renal biopsy using light, electron, and immunofluorescence microscopy have produced an evolutionary change in the practice of renal medicine. The knowledge gained from the study of live renal tissues has enabled clinicians to deliver better medical care to patients with renal diseases. To attain an understanding of renal pathology—particularly expertise with the delicate electron microscope—and of the various technical and medical skills used to prolong uremic patients' lives by dialysis and transplantation requires considerable ability and time; consequently the practice of nephrology (renal medicine) has emerged as a separate subspecialty.

INDICATIONS AND CONTRAINDICATIONS OF RENAL BIOPSY

Numerous papers have been published concerning indications and contraindications of renal biopsies. There are differences and similarities of opinions about the necessities of tissue diagnoses in the practice of nephrology. Nevertheless, there is consistent agreement that renal biopsy is indicated in continuing or persistent renal parenchymal disease, especially when the diagnosis and prognosis are not evident from clinical studies.

Broadly speaking, renal biopsy study is of some value in every renal parenchymal disease, and there is no clinical finding or laboratory test which can replace histopathological evaluation. Before elaborating the indications for a renal biopsy it is helpful to outline some common clinical and laboratory criteria that should facilitate recognition of renal parenchymal disease (Table 2-1).

INDICATIONS OF SINGLE BIOPSIES

Renal biopsies are most commonly performed in chronic renal parenchymal disease. In acute renal parenchymal disease, the overwhelming clinical evidence and the rapid recovery of many patients make a renal biopsy unnecessary. In general, the indications for renal biopsy according to clinical needs can be broken down into (1) definitive, (2) less definitive, and (3) controversial. Definitive indications include (a) nephrotic syndrome in the adult; (b) asymptomatic persistent proteinuria (24-hr proteinuria greater than 1.0 g); (c) recurrent microscopic or gross hematuria when radiological and urological investigations have failed to demonstrate tumor or cystic disease of the kidneys, or any abnormalities in the urinary conduits; (d) acute glomerulonephritis in the adult; (e) rapidly progressive renal failure; and (f) manifestations of original renal disease in the transplanted

Table 2-1
Clinical and Laboratory Criteria of Renal Parenchymal Disease

	Acute renal parenchymal disease	Chronic renal parenchymal disease
Symptoms	Sudden onset, bilateral backache, oliguria, gross hematuria, history of sore throat, history of drug intake	Insidious onset, asymptomatic, or swelling of the body
Findings	Normal, slight edema, hypertension	Normal, moderate to marked edema, hypertension
Urinalysis	2+ to 3+ proteinuria, microhematuria (too numerous to count), pyuria	4+ proteinuria common, often mild microhematuria (10–20 RBC/HPF, may be intermittent)
24-hr proteinuria	<3.5 g usual	>3.5 g common
Blood chemistry	Normal or mild elevation of urea nitrogen and creatinine	Often normal but mild elevation of urea nitrogen not uncommon

kidney. The less definitive indications are as follows: (a) nephrotic syndrome in children; (b) acute glomerulonephritis in children; (c) transplant rejection; and (d) drug-related nephropathy. The controversial items are (a) acute renal failure, (b) chronic renal failure, and (c) essential hypertension.

Among these, the nephrotic syndrome is the ideal indication for renal biopsy, the sole purpose of the renal biopsy in the nephrotic syndrome being to identify the condition or conditions which are amenable to treatment. This subject is dealt with more detail in the chapter on the nephrotic syndrome. It should be emphasized here, however, that lipoid nephrosis or the idiopathic nephrotic syndrome, which is responsive to corticosteroid therapy and constitutes a curable renal disease, may be missed unless a renal biopsy is performed.

It has been well established that lipoid nephrosis is the leading cause for the vast majority (80–85%) of cases of the nephrotic syndrome in early childhood. Therefore, the prevalent consensus among most pediatric nephrologists is not to do routine renal biopsies in young nephrotic children until they have received a 6-week course of corticosteroid therapy. Some believe that this should be the rule for nephrotic children younger than 5 years of age, whereas nephrotic children more than 5 years old at onset should have renal biopsies performed before instituting corticosteroid therapy. Lipoid nephrosis accounts for 20 to 25% of the adult nephrotic syndromes; it is similar to juvenile cases in its response to corticosteroid therapy. This group of nephrotic adults must be distinguished from those with membranous glomerulonephritis, which is less responsive or nonresponsive to corticosteroid therapy. When a cause for nephrotic syndrome is clinically evident (e.g., diabetes mellitus), renal biopsy may not be indicated except for the purpose of teaching or other scientific interest. The latter pertains to the demonstration of associated renal disease, e.g., membranous glomerulonephritis in a small percentage of cases.

The need for renal biopsy in acute glomerulonephritis and in acute oliguric renal failure has remained a controversial subject. Specific reasons are cited here to bridge the disagreement and to convince nephrologists of the need for renal biopsy studies in these two conditions. First, a number of adults with features of acute glomerulonephritis are found to have rapidly progressive glomerulonephritis or necrotizing glomerulonephritis associated with Wegener's granulomatosis. Both of these pathological processes, which are almost always associated with rapid and irreversible deterioration of renal function, must be discovered early in order to prepare these patients for dialytic treatment or immunosuppressive therapy, or both. Second, glomerulonephritis in thrombotic thrombocytopenic purpura may be benefited by splenectomy, corticosteroid therapy, or immunosuppressive drugs.

Finally, the vast majority of cases of acute renal failure are due to acute tubular lesions or necrosis (ATN), and the lesion is generally reversible. When a patient with ATN does not show signs of recovery after 2 to 3 weeks of conservative or dialytic therapy, a renal biopsy must be done to identify the underlying renal pathology, and especially to rule out irreversible pathological processes, e.g., rapidly progressive glomerulonephritis or renal cortical necrosis.

The main purpose for tissue diagnosis in persistent asymptomatic proteinuria is to determine the anatomic pathology of the kidney, the probability of its response to available therapy, and the consequences of that therapy. It is difficult to obtain approval for renal biopsy study in essential hypertension. If there is some evidence to suspect glomerular disease, e.g., heavy proteinuria, renal biopsy is definitely indicated. There are a few studies which have suggested that electron microscopy studies of arterial vessels, along with the other component parts of the kidney, may assist in assessment of the progression of hypertensive renal lesions.

CONTRAINDICATIONS

Among the various contraindications enumerated in current textbooks and periodicals, the solitary kidney and an irreversible bleeding diathesis, in my experience, must still be considered as absolute contraindications. Many of the relative contraindications, such as tumor, cysts, or infection, should not always preclude the biopsy procedure if a definitive indication exists which warrants the additional risk.

Renal biopsy is generally safe after a prolonged peritoneal dialysis or after two to three hemodialyses in cases of acute or chronic uremia, a condition that should no longer be regarded as a contraindication. Similarly, if there is a rationale for renal biopsy study in hypertensive patients, it should not be withheld because of the fear of bleeding. Excessive bleeding can be prevented by reducing the blood pressure to normal or near normal before and during the biopsy and for at least 72 hr following the procedure.

COMPLICATIONS AND SIGNIFICANCE

Mild microscopic hematuria and mild back pain for 24 to 48 hr postbiopsy are encountered in almost all patients. When modern techniques to localize the kidney are used, gross hematuria and passage of blood clots occur in approximately 5% of adults or children. A variety of serious complications have been reported following percutaneous renal biopsy, most or all of which are very rare. The serious complications are as follows:

1. Massive hemorrhage from the biopsy site, accompanied by a fall in hematocrit, occurs in 0.5 to 1% of adults. The incidence may be higher in children, 2.5 to 3%. Bleeding almost always stops with rest and blood transfusions. Nephrectomy has been seldom necessary to control bleeding.
2. The complication of perirenal or retroperitoneal hematoma occurs more commonly than we appreciate clinically. In many instances this complication does not constitute a serious clinical problem and thus is seldom reported. In some series it is reported to be no more than 0.4% of the total number of patients

undergoing renal biopsies. In children, it may occur in up to 1%. Oozing of blood continues after the biopsy so that from 3 to 7 postbiopsy days, a patient may exhibit the severe backache, hematuria, mild elevation of temperature, and guarding of abdominal muscles which signal this complication. The diagnostic aids are a fall in hematocrit and obliteration of psoas shadow on a flat film of the abdomen. The treatment includes bed rest, intravenous fluids, and analgesics. If there is evidence of infection, antibiotics may be used. A urologist should be consulted for possible urologic interventions.

3. When arteriovenous fistula develops, gross hematuria may persist for 10 days or more. A selective renal arteriogram should be obtained that will demonstrate filling of the renal vein simultaneously with the arterial phase. Aminocaproic acid may be effective in arresting bleeding.

4. Among other rare complications which should be mentioned are the biopsy injuries to the liver, spleen, bowel, pancreas, and aorta.

5. Death is now virtually unheard of with the introduction of Franklin's modification of the Vim-Silverman needle and its use in the hands of experienced operators. For example, in a series of 2500 biopsies performed by Lange and Tresser (1974) no deaths were reported.

It should be remembered that serious complications seldom occur at the hands of experienced operators and in cooperative patients. There are, however, no exceptions to certain contraindications, and, therefore, the operators must make proper patient selections and exercise all usual precautions (preoperative and postoperative) to avoid unhappy postbiopsy events.

PITFALLS OF PERCUTANEOUS (CLOSED) BIOPSIES

The rate of success in obtaining a representative sample of kidney (at least five glomeruli in the light microscopy portion of the tissue) varies from series to series. In any large series the attempt is successful in approximately 90% of cases (e.g., Muehrcke *et al.*, 1955; Lange and Tresser, 1974). A third large series by Carvajal and associates (1971) reports about 82% success, considering the presence of at least six glomeruli as a representative sample. Many small series have reported an 80 to 85% rate of success. It is my impression that the success rates have improved slightly in the last few years.

TECHNIQUES OF PERCUTANEOUS BIOPSIES

A detailed description of the different techniques used in performing a renal biopsy is limited by the space available herein. However, the different types of biopsy techniques and the merits and demerits of different techniques may be stated briefly.

1. Blind technique of Muehrcke *et al.* (1955). A thorough physical exami-

nation of the patient is essential in order to rule out hepatosplenomegaly and to avoid laceration of these organs during the biopsy procedure. An intravenous pyelogram (IVP) is a prerequisite step. In the IVP a location (X) is selected at the lower pole of the kidney; measurements are made on the IVP from the corresponding spinous process and iliac crest to the point (X). These measurements are then depicted at distances from the highest point of the iliac crest and the corresponding spinous process by drawing lines with an aqueous solution of crystal violet on the patient's back (prone position). The right side was the site of choice for these authors. After cleaning the side of the back with antiseptic solutions (tincture of iodine followed by 70% alcohol), and anesthesia of the biopsy site with 1% procaine given by intradermal injection, the needle is introduced through the skin and subcutaneous tissue, after which advancement of the needle is made gently, with inspiration only, until it reaches the kidney. The kidney is located by wide swinging of the needle to and fro over half a circle during deep breathing. The "feel" of the kidney through gentle probing of the needle is the most important key to the success of biopsy. This "feel" can only be acquired by experience and with time. Undue pressure on the kidney associated with overenthusiastic attempts to obtain a piece of renal tissue must be avoided. This is, however, minimized as the operators continue to gain experience. This blind technique of renal biopsy has been practiced throughout the world and thousands of successful renal biopsies have been performed by numerous individuals.

2. A modification of the technique of Muehrcke and co-workers has been described by Colodny and Reckler (1975), who prefer using the left kidney. This technique is similar to the preceding one, except that only one measurement is made—the distance between the lower pole of the kidney and the opposite vertebral spinous process in the IVP. This measurement is depicted by painting the skin with the patient in the prone position. The rate of success with this modified technique reached 85%.

3. From the latter part of the 1960s and through the 1970s, renal biopsy has been done most commonly through direct visualization of the kidney via the television screen. In this technique, a preliminary IVP is not required. The contrast material (0.5 ml/lb body weight; average: 60 ml) is added to a bottle containing 500 ml of intravenous fluid which is infused slowly until the kidney to be biopsied is visualized. If the patient has azotemia, a double dose of the contrast material (1 ml/lb body weight) is added to the same amount of fluid.

The advantage of this technique is that the needle is inserted into the lower pole of the kidney under direct visualization; the potential disadvantage is the exposure of the patient and the operator to excessive radiation. For example, the radiation dose received by the patient in an IVP is 0.468 rads per film but 4.4 rads per examination. A single 10- or 15-min film alone or both films should provide a good nephrogram suitable for biopsy. This gives a radiation exposure of no more than 1 rad. In contrast, the radiation dose for a single fluoroscopy with the image intensifier for each 90-sec period is less than 3 rads. Therefore, the risk to the patient of excessive radiation is likely should several fluoroscopies

become necessary to visualize the kidney. This risk increases in particular with azotemic patients. The advantage of this technique is the greater rate of success, which may approach 100% in the hands of experienced operators. The percentage of tissue samples obtained that prove to be adequate is not, however, different from that of the blind technique. We have found no difference between the two techniques in our own practice with respect to the rate of success and the adequacy of the sample. However, the rate of gross hematuria following biopsy is undeniably less if direct visualization is employed. Disadvantages of the direct visualization technique are the necessity of a fluoroscope suite, the complex technical operation around the intensifier-television chain, and the necessity of having one or several assistants.

4. Renal scan prior to renal biopsy. This was performed at the University of Chicago Hospitals and Clinics, in which kidneys were localized 1 hr after an injection of 1.0 mCi of [^{99m}Tc]-Fe-ascorbic acid complex under the gamma camera. In a series of 377 patients on whom renal biopsies were attempted, adequate tissue was obtained in 82% of the patients. With further sophistication of the technique, adequate tissue was obtained in 99% of the patients.

5. Ultrasonography (ultrasound) for renal localization. The patient is placed in the prone position on a radiographic wedge. A B-mode ultrasound scanner with simultaneous A-mode capability is used to record transverse echograms on a storage oscilloscope, with the iliac crest as a reference point. The medial and lateral borders of the kidneys are marked directly on the back; the upper and lower poles are marked during normal and full respiration. A longitudinal echogram is recorded for each kidney, and the depth of the lower pole is measured directly from the echogram. The closed renal biopsy is performed in exactly the same position with use of standard techniques. Renal tissues were obtained in 29 of 30 patients in whom biopsies were attempted. The localization method is fast, inexpensive, accurate, and noninjurious. The additional advantages are the lack of a need for contrast material and the elimination of the risk of radiation.

COMPARISON BETWEEN OPEN AND PERCUTANEOUS (CLOSED) RENAL BIOPSIES

Open renal biopsy has been advocated as a more advantageous procedure than closed renal biopsy. The open biopsy technique remains the only way to secure renal tissue when a percutaneous renal biopsy has failed, when a biopsy is indicated in an uncooperative patient, or when a closed biopsy involves a high risk of bleeding in azotemic patients. The greatest advantage of the open technique is the virtually complete assurance that adequate tissue will be obtained. Although there are no reported mortalities by this technique, complications due to general anesthesia and the surgical procedure are not rare. For example, we have seen postoperative paralytic ileus which caused much misery and prolongation of the hospital stay of a patient who underwent open renal biopsy. In a series of open wedge biopsies in 61 children, although adequate tissue was obtained in all patients, minor postoperative complications were found in 10%

of the patients. Therefore, the rate of complication in open biopsies did not differ from that in closed biopsies.

We believe that the superiority of the techniques in obtaining adequate tissue for optimum studies depends greatly on the experience of the operators.

In the hands of experienced operators a percutaneous biopsy is a safe and easy method for obtaining a large piece of kidney for complete histological studies. This opinion is consistent with those of others. The different percutaneous biopsy techniques have been summarized in Table 2-2.

CLINICAL JUSTIFICATIONS FOR THE COMPLETE MORPHOLOGICAL STUDIES OF RENAL BIOPSIES

There is no longer any need to emphasize the values of histopathological studies of the kidneys in the clinical practice of nephrology. These values are illustrated in Fig. 2-1. Complete morphological studies of the kidneys using all three microscopy techniques should be done in order to provide an exacting anatomical diagnosis and to aid the determinations of the etiology and pathogenetic mechanisms of the disease processes. This information should guide the practicing physician in instituting appropriate therapy and in assessing the course and prognosis of the disease processes. Knowledge of the irreversible or progressive renal lesions would enable the primary care physician or the nephrologist to prepare the patient for dialytic and transplantation therapy. Similarly, awareness of the pathogenetic mechanisms helps to estimate the risk of a potential grafted kidney for acquisition of the original disease process. Immune complex glomerulonephritis may recur in the donor kidney, but the incidence is low. Therefore, a small risk should not preclude the patients with immune complex glomerulonephritis from receiving kidney transplants. In contrast, recurrence is common in the recipients with anti-GBM-antibody glomerulonephritis, especially

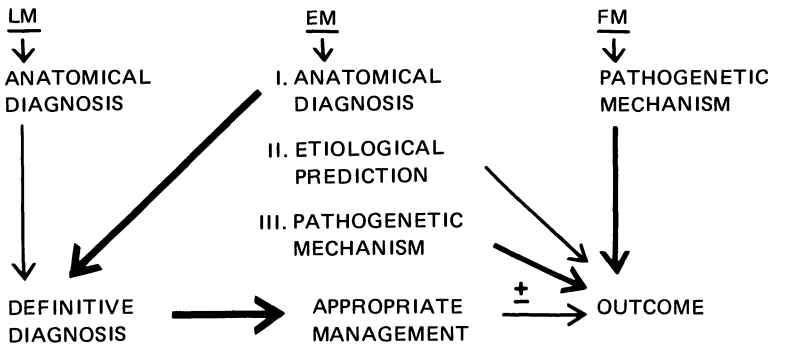

Fig. 2-1. This illustrates the diagnostic, therapeutic, and prognostic values of renal biopsy studied by three different types of microscopy techniques. LM, Light microscopy; EM, electron microscopy; FM, fluorescence microscopy. A thick line represents a greater contribution, a thin line, a lesser contribution.

Table 2-2
Accuracy[a] of Renal Localization Procedures

Author	Accuracy	Technique
Kark and Muehrcke, 1954	47 of 50 (94%)	Pyelogram
Kark *et al.*, 1958	401 of 500 (80%)	Pyelogram
Brun and Raaschou, 1958	162 of 243 (67%)	Not stated
Lindham *et al.*, 1967	114 of 150 (76%)	Pyelogram or plain abdominal film
Lusted *et al.*, 1958	9 of 10 (90%)	Image amplifier and pyelography—direct vision of needle progress
Ginsburg *et al.*, 1962	30 of 37 (81%)	Image amplifier and pyelography
Kark and Buenger, 1966	All work (87%)	TV image intensifier and infusion IVP—done in normal room illumination—specificity of cortex vs. hilum
Haddad and Mani, 1967	22 of 23 (96%)	TV image intensifier and infusion IVP
Junghagen *et al.*, 1968	57 of 68 (84%)	TV image intensifier and arteriography—not limited by uremia
Kark, 1968, appendix by Welt	Survey (89.9%)	TV image intensifier and infusion IVP—21 nephrology centers—data representing 8081 biopsies, 1398 done under fluoroscopic control
Fajers *et al.*, 1970	54 of 66 (82%)	TV image intensifier and arteriography—not limited by uremia
Kaplan *et al.*, 1970	31 of 32 (97%)	TV image intensifier and pyelography—use of a disposable needle
Telfer *et al.*, 1964	9 of 11 (82%)	10 μCi of [^{203}Hg]chlormerodrin with portable scintillation counter
Baum *et al.*, 1966	6 of 7 (86%)	200 μCi of [^{197}Hg]chlormerodrin with rectilinear scanner
Forland *et al.*, 1967	16 of 17 (94%)	1 mCi [^{99m}Tc]-Fe-ascorbate with rectilinear scanner—applicable to children
Reese and Joshi, 1968	30 of 30 (100%)	100 μCi of [^{203}Hg]chlormerodrin with rectilinear scanner
Zimacek *et al.*, 1970	37 of 40 (92%)	3.5 Ci/kg [^{197}Hg]chlormerodrin with rectilinear scanner or gamma camera
Berlyne, 1961	18 of 20 (90%)	Ultrasound flow detector
Tully *et al.*, 1972	343 of 377 (91%)	Renal scan prior to renal biopsy

[a] Accuracy defined as recovery of tissue of diagnostic value as defined previously.
Source: Reproduced by the kind permission of Tully *et al.* (1972).

if a donor kidney is grafted without removal of recipient kidneys, too early after bilateral nephrectomies, or before disappearance of circulating anti-GBM antibody.

Since facilities for electron and immunofluorescence microscopy studies are limited, some indices from the light microscopy studies have been mentioned which should prove useful in the clinical care of patients with glomerulonephritis. Predominant endocapillary proliferative and exudative changes have greater chance of reversibility and recovery, whereas a predominant epithelial cell proliferation with florid crescent formation is more likely to be an irreversible process. The finding of normal renal histology in the nephrotic syndrome indicates a greater likelihood of response to corticosteroids; on the contrary, it is not yet

established that membranous glomerulonephritis will respond to drug therapy.

Once the results of complete morphological studies of the kidneys are at hand, the clinician should be in a position to prescribe therapy rationally (drugs or transplant) and to determine the course of the disease process on a scientific basis. Since electron and immunofluorescence microscopy facilities and people skilled in handling the equipment are not universally available, a thorough and careful study of relatively thin sections (2–3 μm) using light microscopy alone may provide sufficient information to allow good clinical care. In addition, the physician must endeavor to obtain adequate information by taking a good history and performing a thorough physical examination. The clinical data, along with the laboratory tests, make the morphological diagnosis more meaningful, and the combination of all of these examinations, including the histopathological study of the kidney, should improve the quality of care received by the individual patient.

ETHICS AND INDICATIONS FOR SERIAL RENAL BIOPSIES

Serial (repeated) renal biopsies have been performed on patients with glomerular diseases by numerous investigators, including the author. The results of some large studies have been reported, although most of the smaller studies have remained unreported. Each of us depends on the clinical features and laboratory studies to obviate the need for renal biopsies whenever possible. In this context, we mentioned earlier that no laboratory test can completely substitute for histopathological evaluation. This theme is applicable to the initial biopsy, which is performed for purposes of diagnosis and institution of therapy, and to the subsequent biopsies used to evaluate morphological changes toward reversibility or irreversibility.

The etiologies of most chronic glomerular diseases remain obscure. It is apparent that some of these chronic glomerular diseases, as well as those with the "wastebasket" classification of end-stage renal disease, emerge from unhealed acute glomerular diseases. Serial histopathological studies, following an episode of acute glomerulonephritis, would allow us to establish or eliminate this disorder as an initiating cause. The progression from acute to chronic disease is more likely to happen, in particular, in adults who often have subclinical diseases and in whom pathological processes do not reverse as often as in children. Similarly, the therapeutic trial of many pharmacological agents warrants repeated histopathological studies in order to evaluate the effectiveness of the drugs against the progression of the pathological process.

Among other indications, repeated histopathological studies appear imperative in the clinical management of membranous glomerulonephritis, especially from the perspective of allowing staging and potential benefits from the use of

therapeutic agents. Possible indications for repeated biopsies then may be enumerated as follows: (1) proteinuria persisting longer than a year following an episode of acute glomerulonephritis in children or adults; (2) patients with glomerular diseases undergoing therapeutic trials; (3) membranous glomerulonephritis; (4) asymptomatic persistent proteinuria which has been found on routine physical examination; and (5) corticosteroid-unresponsive nephrotic children, especially when the first biopsy is nondiagnostic. Repeated renal biopsies seem to be ethically justified in these clinical situations. A question often is asked by the patients as to whether or not more damage will be inflicted on the kidney by repeated biopsies. There is no good data to indicate that repeated biopsies are harmful to overall renal function. Patients should be reassured that rebiopsies should not be more harmful than the damage already present in the kidney. Also, it should be mentioned that the incidence and severity of postbiopsy complications in each subsequent biopsy are no greater than for the initial biopsy.

PART 2
METHODS OF FIXATION, PROCESSING, AND STUDY

INTRODUCTION

Ideally every renal biopsy specimen should be studied by using light microscopy, electron microscopy, and immunofluorescence microscopy. Centralized electron microscopy (EM) and immunofluorescence microscopy (IFM) units have been established to render services to the physicians in peripheral or community hospitals.

Prior to each renal biopsy the author's laboratory supplies to the operators a kit furnished with three bottles containing 10% formalin, 4% glutaraldehyde, and 0.9% saline, respectively. The kit contains ice to keep the solutions cool.

The core of renal tissue obtained through percutaneous biopsy is divided into three portions, one portion for each of the three studies. From the biopsy core of an average length of 20 mm (2 cm), an approximately 2-mm piece is cut at each end of the core and immersed in the glutaraldehyde in 0.1 M phosphate buffer, a 3-mm piece at one end is cut and placed in the saline, and the remaining 13-mm piece is placed in the formalin. The bottles are kept cold until they are delivered to individual laboratories.

Light Microscopy Study

The formalin-fixed tissue is dehydrated and embedded in paraffin. From the paraffin blocks, sections of 3- to 4-μm thickness are cut, stained with hematoxylin-eosin (H & E) and periodic acid-Schiff (PAS), usually with periodic acid-

methylenamine silver (PAMS) or silver stain (Jones, 1951) and Gomori's trichrome. The H & E-stained sections render nuclear structures dark purple or blue, and impart a pink appearance to the cytoplasm and cytoplasmic structures. PAS imparts better visualization of the basement membranes of the glomeruli, tubules, arterial vessels, glomerular mesangium, tubular microvilli, and juxtaglomerular apparatus (JGA); Gomori's trichome makes collagen fibers and fibrous tissue prominent. In selected biopsies, congo red or crystal violet stain for suspected amyloid, and Hart and Verhoeff's stain* for demonstration of elastic tissue are applied.

It is to be remembered that for the purpose of overall assessment H & E and PAS stains are adequate. If an adequate EM facility is available, the battery of LM stainings is unnecessary.

Electron Microscopy Study

The tissues fixed in glutaraldehyde are subsequently treated in the following order:

Postfixation: (1) 1% osmium tetroxide (OsO_4) buffered with PO_4 at pH 7.2 for 1 hr (first postfixation); (2) 10% PO_4-buffered formalin at pH 7.2 for 30 min (second postfixation).

Dehydration: Tissues are dehydrated with the following materials: (1) graded alcoholic solutions from 70% through absolute, each for 15 min; (2) propylene oxide—two changes, each for 15 min; (3) propylene oxide and embedding media 1:1 ratio—two changes, each for 15 min.

Embedding: Tissues are embedded with Spurr low-viscosity embedding media in Beem capsules (blocks) and polymerized in the oven at 60° for 12 hr.

From the blocks, 0.5-μm so-called thick sections are cut, using Porter–Blum microtome MT I or II, collected on glass slides, and stained with 1% methylene blue-azure II (equal volume of each: Mallory's stain) for 1 min, washed with distilled water, and examined with a light microscope. When a particular structure under consideration for study, e.g., glomerulus or arterial vessel, is found, the block is trimmed further, and thin sections (300 Å) are cut from the block. The thin sections are collected on copper grids, and occasionally on gold grids. The copper grids are stained with uranyl acetate and lead citrate (UA + LC) by the standard method. The gold grids are stained with either 1% PAMS or PAS or silver tetraphenyl porphyrin sulfonate (STPPS), or both.

SILVER IMPREGNATION (PAMS OR PAS) TECHNIQUE

Gold grids (300 mesh) containing the sections are floated on aqueous 1% periodic acid for 10 min and washed twice in distilled water. The grids are then

* Hart and Verhoeff's stain is a reasonable technique for quick assessment of elastic tissue. Since it may also stain basement membrane or collagen fibers (Fig. 2-2), it is not the optimum technique for overall quantitation of elastic tissue.

Fig. 2-2. A thick dark band on the inner aspect of the small artery is seen. This dark band consists of basement membrane and elastic tissue (Hart and Verhoeff's stain, ×320).

floated on methylenamine silver solution at 60°C for 15 min, washed in 3% sodium thiosulfate for 2 min, and finally washed twice in distilled water. Stock methylenamine silver solution is prepared with 40 ml of 3% hexamethylenamine and 5 ml of 10% silver nitrate, and after mixing, 5 ml of 2% sodium borate is added to the solution. The working solution is prepared by adding 8 parts of distilled water to 2 parts of stock solution. If background precipitation occurs, washing time in sodium thiosulfate is increased.

SILVER TETRAPHENYLPORPHYRIN SULFONATE STAINING TECHNIQUE

A fresh solution of STPPS is made each time by dissolving 10 mg of commercially available dry powder (Alpha-Electron Microscopy Inc., Rockville, Maryland) in 5 ml of double-distilled water. The fresh solution is cherry red in color

and has a neutral to slightly alkaline pH. Individual drops of this solution are placed in a Petri dish. The gold grids holding the sections are laid face down on each drop, and the entire preparation is placed in an oven at 60°C for 15 min. After removal from the oven, the grids are washed in three changes of double-distilled water and dried.

Detailed information concerning dehydration and embedding, thick and thin sectioning, and staining with UA + LC procedures is available in any of the standard textbooks dealing with the techniques of electron microscopy. Two books which have proved to be valuable in my own practice are Hayat (1970) and Kay (1965).

The staining with UA + LC is sufficient for routine evaluation of renal pathology. The specific stains, i.e., PAMS or STPPS, are done for detailed studies of certain structures. The values of these specific stains are outlined and illustrated in various figures in this book. In addition, the importance of these stains for evaluations of the morphological abnormalities of the different components of the kidney is demonstrated in the appropriate chapter.

VALUES OF SPECIAL EM STAINING TECHNIQUES

PAMS Staining Technique

When the PAMS-stained grids are studied by EM, the basement membrane, mitochondria, and endoplasmic reticulum appear as conspicuous structures. With this staining, detailed evaluation of the mitochondria can be made (see Fig. 5-16). Therefore, this technique has been found to be very valuable in the assessment of tubular damage, e.g., tubular necrosis, interstitial nephritis, and pathology of the transplanted kidney. It may also be valuable in semiquantitative analysis of the basement membrane thickness and basement membrane-like materials.

STPPS Staining Technique

This technique produces specific staining of elastic tissue, yielding electron-dense material (EDM) at tissue sites corresponding to the electron-lucent material observed in UA + LC-stained sections (Fig. 2-3). EDM is observed in all vessels studied. Variable quantities of EDM are demonstrated in the small arterioles (see Fig. 5-26) between individual smooth muscle cells, the outer perimeter of larger arterioles, and small arteries. Occasionally EDM is found in basement membrane of the peritubular capillaries. In general, more elastic tissue is observed in the STPPS-stained sections than that suggested by the quantity of electron-lucent material found in UA + LC-stained sections (see Chapter 5). This difference is most marked in spontaneously hypertensive rats and severely hypertensive patients. When this staining technique is utilized, the different types of arterial vessels may be readily distinguished.

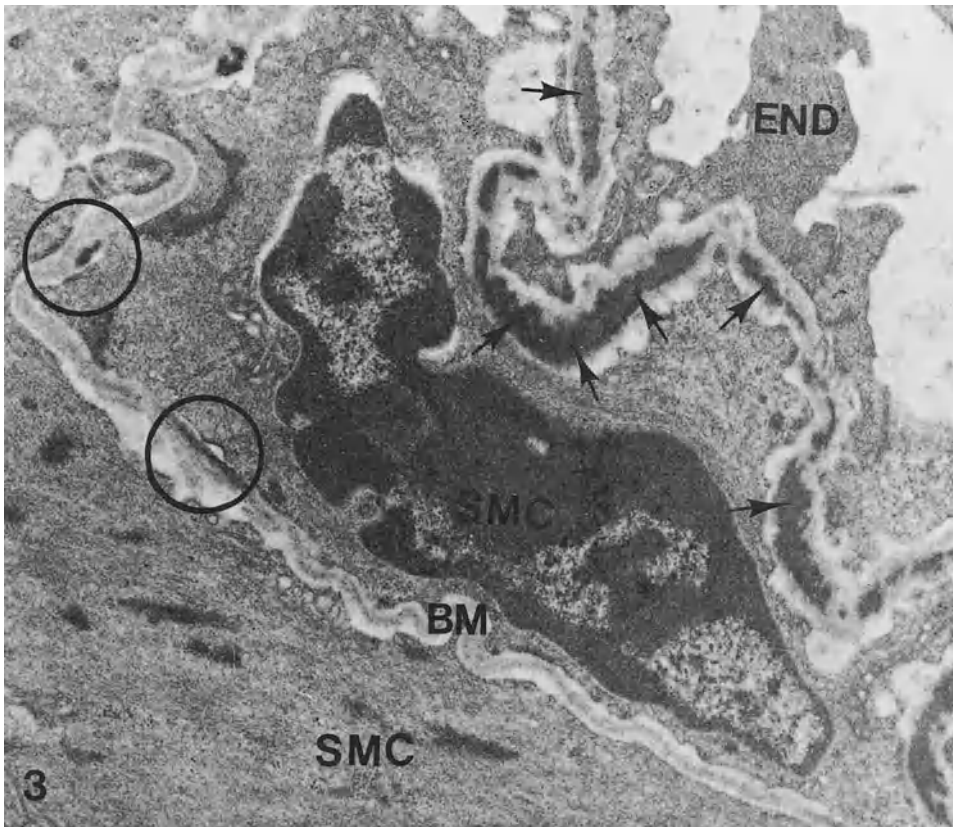

Fig. 2-3. An electron-dense band of elastic tissue (arrows) is seen in the center of the basement membrane between endothelial cell (END) and smooth muscle cell (SMC). Small fragments of electron-dense elastic tissue (circles) are seen within the basement membrane (BM) between SMC (STPPS, ×28,000).

SUMMARY

1. Numerous published series have confirmed that percutaneous renal biopsy is a safe and useful technique in the practice of nephrology.

2. Renal tissue should be studied using light, electron, and immunofluorescence microscopy. If facilities for electron or immunofluorescence microscopy are not available, a situation not uncommon in peripheral hospitals, it should be arranged to have the patient biopsied at a medical center with suitable facilities. This would ensure complete study of the renal tissue.

3. Arguments still exist concerning the necessity of renal biopsy studies for some particular types of renal diseases. In general, this study is of real value in nephrotic syndrome, acute glomerulonephritis, and rapidly progressive renal failure of unknown etiology. This study is far more important in adults than in children.

4. The choice of a technique for the biopsy procedure depends greatly on the individual physician. However, the blind technique, renal scan method, and ultrasonography are safer, especially with respect to irradiation of the patient, than is the direct visualization technique.

5. Postbiopsy complications are generally mild and cause no anxiety. Occasional serious complications, e.g., prolonged hematuria or perirenal hematoma, do occur and create much apprehension in the minds of patients and physicians. However, they are scarcely ever life threatening.

6. Electron microscopy study is not always necessary, but a portion of the tissue should always be fixed for EM study. If none or few glomeruli are found in the portions for LM and IFM studies, or if the diagnosis obtained by these two studies is obscure, EM study must be done in order to obtain information about detailed morphological abnormalities and reach a working diagnosis.

7. A thorough study of the biopsy material is needed to justify institution of appropriate therapy and assessment of the course of the disease process.

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3 Clinical Assessment of the Etiology and the Pathological Activity of Glomerulonephritis

The etiology and pathological activity of glomerulonephritis (GN) can be assessed by three different methods: (1) history and physical examination, (2) laboratory tests and their applications to determine the etiology of glomerulonephritis, and (3) laboratory tests to indicate the activity of the pathological process.

ASSESSMENT OF THE ETIOLOGICAL FACTORS RELATIVE TO GLOMERULONEPHRITIS

In only a relatively small number of cases of glomerulonephritis, characteristic histopathological features, such as typical "humps" which strongly suggest poststreptococcal glomerulonephritis, have been demonstrated. In the majority of cases, however, a paucity of classical histopathological features causes difficulty in characterizing the type or the etiology of a given case of glomerulonephritis. Consequently, in the evaluation of patients with glomerulonephritis, morphological diagnosis must be supplemented by a complete history, a thorough physical examination, and appropriate laboratory tests.

PATIENT HISTORY

The following information should be obtained:

1. Inquire about a preceding sore throat or pharyngitis, and symptoms such as bilateral backache, blood in the urine, or small amount of urine, which are suggestive of acute glomerulonephritis. If a throat culture had been done earlier, and an organism was isolated, the laboratory should be contacted for the results.

Throat culture results become even more important if a patient has recurrent episodes of the nephritic symptoms. It is important to realize that recurrent poststreptococcal glomerulonephritis, although rare, does occur, but is unlikely to be due to the same type of streptococcus as the original attack. In temperate climates, pharyngitis commonly precedes acute glomerulonephritis.

2. It is important to note any drugs that may have been prescribed for sore throat(s), whether or not penicillin was ever used, and whether it appeared to afford prompt relief. This may indicate that a group A beta hemolytic streptococcus was the offending organism.

3. Ask also whether or not the patient had a preceding skin infection, such as scabies, impetigo, or pyoderma. Check for culture of the skin and treatment with antibiotic(s). In my experience, as well as in the experience of others, acute glomerulonephritis commonly follows scabies or impetigo in tropical countries, e. g., India and parts of Asia, Central America, and sporadically in other countries. Acute glomerulonephritis following impetigo among Indian children has been reported in the southern United States.

4. Check for a history of arthralgia, arthritis, intermittent fever, skin rash, and sensitivity to sunlight, i.e., skin blisters developing after exposure to sunlight. Two-thirds of all patients with lupus glomerulonephritis give a history of systemic manifestations at the time renal disease is discovered.

5. Question the occurrence of abdominal colic, bloody diarrhea, hemorrhagic rash on the inferior extremities followed or accompanied by oliguria or bloody urine, or both (suggests Henoch–Schonlein purpura).

6. Investigate for a history of chronic heart disease for possible complication of bacterial endocarditis, surgical procedures for the drainage of visceral abscesses, spiking fever, and lymphadenopathy (lymphoma, sarcoidosis, syphilis, etc.).

In addition, look for drug abuse, e.g., when heroin addicts are questioned; intake of drugs, e.g., penicillamine, gold for arthritis; and the presence of infected ventriculoatrial shunts in hydrocephalic infants or children.

CLINICAL LABORATORY TESTS

The following laboratory tests are performed: (1) urinalysis; (2) culture of the throat and/or skin lesion; (3) complete blood counts, including platelet count and reticulocyte count; (4) 24-hr urine for quantitative protein and creatinine concentrations; (5) serum creatinine and urea nitrogen concentrations; (6) streptococcal antibodies; (7) tests for systemic lupus erythematosus; (8) serum complement and its components; (9) serology for syphilis; (10) erythrocyte sedimentation rate; (11) blood smear for malaria parasites (when indicated); (12) blood cultures (aerobic and anaerobic); (13) electrophoresis of serum and urinary proteins; (14) serum cryoglobulins; (15) sickle cell preparation; (16) hemoglobin electrophoresis; (17) coagulation studies: prothrombin time (PT), partial throm-

bloplastin time (PTT), plasma fibrinogen, etc.; (18) radiological studies: chest film, intravenous pyelogram, etc.; (19) protein clearance studies for selectivity of proteinuria; (20) fibrin split products in the urine; and (21) viral antibody titers. All these tests might document the setting in which glomerulonephritis occurs as a complicating event. In addition, some of these tests (see later) should aid assessment of the activity of the pathological lesion.

The clinical laboratory tests are now described separately, with the exception of those listed as items 4, 5, 15, and 16, which are omitted owing to space limitations.

Urinalysis

The value of examination of the spun urinary sediment using light microscopy has been emphasized in the past. Growing experience suggests that examination of the urinary sediment is useful in predicting a lesion in component part(s) of the kidney; for example, many erythrocyte and erythrocyte casts imply acute glomerular lesions alone, such as acute glomerulonephritis, glomerular necrosis, or such arteriologlomerular lesions as malignant hypertension or polyarteritis. The presence of many leukocytes and leukocyte casts strongly supports a diagnosis of acute pyelonephritis. Hyaline casts may be found in any glomerular disease regardless of the etiology and severity of the disease process; granular casts are found most commonly in any chronic glomerular disease irrespective of the etiology. A combination of mixed cellular elements and a variety of casts popularly called telescoped urinary sediment has been considered by many as a characteristic feature of lupus nephritis. The popularity of this finding as an aid in the diagnosis of lupus nephritis has decreased over the years since this type of urinary sediment has been observed in a variety of acute and chronic glomerular diseases.

Urinalysis provides slight or no assistance in the diagnosis of acute tubular lesions or acute interstitial nephritis. In support of this notion, several reports are cited which will demonstrate the limited values of urinalysis in the diagnosis of tubular and tubulointerstitial diseases. Abnormal urinalysis was found in only 4 of 30 (13%) patients with acute tubular necrosis reported by Lewers and associates (1973). Even so, the abnormalities were mild in three of four patients, for example, 2 to 4 RBC (red blood cells) per HPF (high-power field). Two had granular casts and one had hyaline casts. Ooi *et al.* (1975) found a few RBC and WBC (white blood cells) in the urinary sediment in all of their patients with histologically proven acute interstitial nephritis. These urinary findings were not different from those in control patients. Nolan and Abernathy (1977) noted microhematuria to frank blood in the urine, and mild to severe pyuria in 15% of patients receiving methicillin treatment. Since methicillin has been involved in the pathogenesis of acute interstitial nephritis, appearance of abnormal urine in a patient who is receiving methicillin (normal urinalysis prior to treatment) certainly warrants renal involvement. This does not, however, indicate acute interstitial nephritis.

In the author's experience the diagnostic urinary sediment is that showing pyuria and bacteriuria. Thus, numerous WBC, clumps of WBC and/or WBC casts along with many motile bacteria indicate active urinary tract infection. In contrast, a few WBC with or without WBC casts, and with or without bacteria, merely suggest chronic undefined renal parenchymal disease, especially chronic tubulointerstitial nephritis. Repeated observations of such a finding in the urinary sediment, in the face of negative urine culture, are often consistent with tuberculosis of the kidney, although this is now a rare disease in the United States.

There is a widespread belief that examination of the urinary sediment is of some value in discerning the etiology of glomerular diseases but there is no direct evidence or established data to support this notion. However, serial urinalyses are useful in clinically judging the activity of a given renal disease. Thus, complete disappearance of cellular elements and casts, and demonstrations of a normal urinalysis on consecutive visits following episodes of acute glomerulonephritis may denote either reversibility or permanent arrest of the pathological lesion. On the other hand, persistent or intermittent appearance of cellular elements(s), especially RBC, and granular casts points toward chronic or persistent glomerulonephritis, e.g., membranous glomerulonephritis, mesangioproliferative glomerulonephritis, or sclerosing glomerulonephritis.

The prospective clinical values of microscopic examination of urinary sediment are shown in Table 3-1.

Culture from Throat and Skin

A throat culture is often negative when the patient presents with acute glomerulonephritis. Group A streptococci are nephrotoxic, and of the M types, type 12 is the most commonly found group A streptococcus associated with acute glomerulonephritis (AGN). In epidemics of acute glomerulonephritis, types 49 and 55 are more commonly isolated, especially when AGN follows pyroderma or impetigo.

Complete Blood Counts and Platelet Count

Hematological abnormalities such as hemolytic anemia, leukopenia, and thrombocytopenia are not infrequent in glomerulonephritis associated with thrombotic microangiopathy, e.g., thrombotic thrombocytopenic purpura (TTP) and systemic lupus erythematosus (SLE).

Streptococcal Antibodies

Antistreptolysin O Antibody

The antistreptolysin O antibody (ASO) titer is very useful in clinical practice especially since it is done in almost all clinical microbiological laboratories. Streptolysin O is a hemolytic substance produced by most strains of group A streptococci. Antibody measured in response to this hemolytic substance is

quantitated and expressed in Todd units. A titer of 160 Todd units or less is considered normal. A mildly elevated single ASO titer merely suggests a past group A streptococcal infection. On the other hand, a rapid rise in titers or a markedly elevated single titer (800–1000 Todd units) indicates active group A streptococcal infection. A high ASO titer is frequently present in acute post-streptococcal glomerulonephritis (60–80%) which follows a sore throat or proven pharyngitis. On the other hand, no more than 25% of acute glomerulonephritis preceded by streptococcal pyoderma demonstrate an elevated ASO titer.

Antistreptohyaluronidase Antibody

Antistreptohyaluronidase antibody (AH) is produced in response to streptococcal hyaluronidase. A titer greater than 1:250 is considered to be elevated. Markedly elevated single titers or rising titers are found in 60% of poststreptococcal glomerulonephritis (PSGN) preceded by sore throat or pharyngitis. AH titer is less reliable than ASO titer, but when used in conjunction with ASO titer, as many as 90% of the individuals with an antecedent streptococcal infection will show an elevated titer to at least one of the two antigens.

Streptococcal Desoxyribonuclease

Streptococcal desoxyribonuclease (also called streptodornase) titer has also been found to be useful in streptococcal pyoderma. This antibody is measured as anti-DNase-B titer. A titer up to 250 units is found in normal subjects. Elevated titers may be found in 70% of PSGN due to streptococcal pyoderma.

All titers are found to be elevated in a small percentage of PSGN preceding skin infections.

Tests for SLE

Lupus glomerulonephritis is not a primary renal disease. The kidneys become involved in the process of sharing the severe immunological brunt of systemic

Table 3-1
Prospective Clinical Values of Urinary Sediments

Index	Value[a]
1. Warning of renal involvement in systemic diseases, e.g., SLE, acute or subacute bacterial endocarditis	3+
2. Suspicion of glomerular disease	3+
3. Suspicion of tubular disease	±
4. Suspicion of drug-induced acute interstitial nephritis	3+
5. Suspicion of severe hypertensive renal disease	2+ to 3+
6. Etiology of glomerular or tubulointerstitial disease	0
7. Persistence (or activity) or reversibility of histological lesion	3+

[a] 0, None; ±, variable; 2+, fair value; 3+, good value.

lupus erythematosus. This disease affects the nuclei primarily, the pathological events being in the nuclei of the endothelial cells. Thus, it is highly probable that the most severe involvement of the kidneys is due to their possession of a greater number of endothelial cells than the other organs in the body.

Lupus Erythematosus (LE) Cell Test

Since this laboratory test is ordered by most physicians, it seems appropriate that the physician be cognizant of the principle of this test. The LE factor, which is an IgG (antibody) in the serum of patients with SLE, reacts with the nucleoprotein in the nuclei of circulating white cells and capillary endothelial cells. The nuclei undergo degenerative changes and are transformed into a homogeneous, structureless mass. This mass is hypereosinophilic and strongly PAS positive (seen as hemotoxylin bodies in histologic section). The nuclei, while undergoing degenerative changes, attract neutrophil leukocytes in the peripheral blood by chemotactic forces. Thus, a phagocyte (neutrophil) with the ingested nuclear material forms what is called an *LE cell.* The LE cell preparation from the peripheral blood appears under the light microscope to be one large cell with large homogeneous eosinophilic material, a thin rim of cytoplasm, and a nucleus compressed to the periphery of the cell. This degenerative cell may be surrounded by many neutrophil leukocytes having edged nuclei and thinned cytoplasm, a characteristic that is called *rosette formation.*

Antinuclear Antibody

The typical antinuclear antibody (ANA) is an antinucleoprotein (anti-DNP). This antibody (LE factor) gives rise to the LE cell phenomenon and is most commonly demonstrated by fluorescence microscopy, but it can be determined by complement fixation technique. This is a more reliable and sensitive test for the diagnosis of SLE, since the LE cell preparation may be negative in 25% of patients with SLE. A measurable amount of the antibody titer is nearly always present in SLE, regardless of the results of treatment or activity of the pathological process.

Anti-DNA Antibody

Anti-DNA antibody is positive in two-thirds of the patients with SLE. Since anti-DNA antibody is seldom positive in other conditions, it has formed the most dependable and sensitive test for diagnosis of SLE. In the experience of some investigators, high DNA binding activity has been confined largely to SLE. In particular, patients with other diseases, including Sjögren's syndrome, lupoid hepatitis, and rheumatoid arthritis, in whom antinuclear antibody is positive, demonstrate DNA activity which is normal or only weakly positive. A negative test for total antinuclear antibodies, however, is strong evidence against a diagnosis of SLE. It is especially important to know that double-stranded anti-DNA

antibody is normal in drug-induced SLE, despite strongly positive antinuclear antibody. A study on DNA binding activity was made on 262 patients with SLE and allied diseases who had positive ANA. The results are shown in Fig. 3-1.

Immunofluorescent Skin Test

Positive staining for immunoglobulins has been observed at the dermal–epidermal junction in punch biopsies from normal-appearing skin in a high percentage of patients with active SLE and renal disease.

Serum Complement and Its Components

It is very common practice to order measurement of the third component (C3) of the serum complement in the diagnostic workup for glomerulonephritis. Normal serum C3 levels vary slightly in different laboratories; with such variation, a value of 123 to 167 mg/100 ml is generally considered normal. Low values may be found in all acute immune complex glomerulonephritis, as well as in chronic immune complex glomerulonephritis with the exception of membranous glomerulonephritis. Serum C3 level is low as long as the disease is active, but tends to return to normal after recovery (e.g., PSGN) or during remission of the

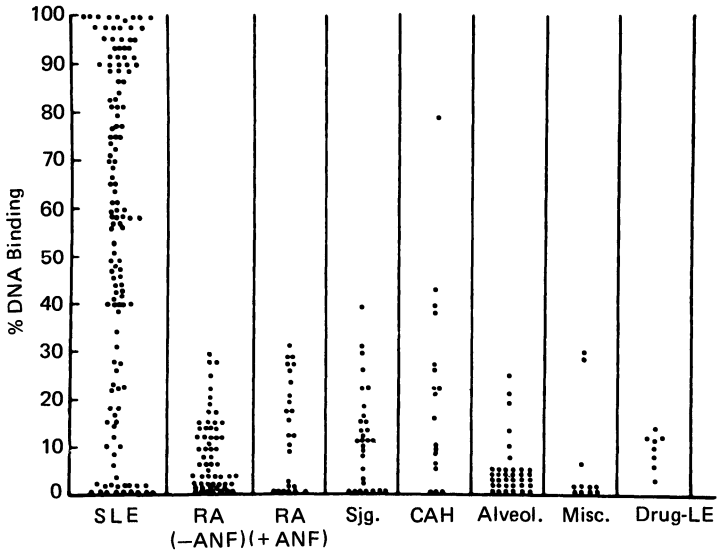

Fig. 3-1. DNA binding activity in 262 antinuclear factor (ANF, equivalent to ANA)-positive sera from patients with SLE and related diseases. A control group of rheumatoid arthritis patients with negative ANF tests is shown in the second column. Values of less than 30% are seen in all but seven of the non-SLE sera. Sjg., Sjögren's syndrome; Alveol., fibrosing alveolitis; Misc., miscellaneous; C.A.H., chronic active hepatitis. From Hughes and Lachman (1975). (Reproduced from the *Ninth Proceeding on Advanced Medicine,* Royal College of Physicians, London, England, by the kind permission of G. R. V. Hughes, M. D., Royal Post Graduate Medical School, London, England.)

disease process (e.g., lupus glomerulonephritis). The exception to this rule is mesangioproliferative glomerulonephritis, in which the serum C3 may remain permanently below normal.

The cause of the low serum C3 in glomerulonephritis is unclear; it was originally thought to be the result of C3 deposition in the glomeruli, but the degree of C3 deposition in the glomeruli does not correlate with the serum C3 level except in mesangioproliferative glomerulonephritis. This serum protein has been found to be more valuable as an index for assessment of the activity of the pathological process than in its function in the evaluation of the disease process. Nevertheless, the finding of low serum C3 complement, accompanied by the presence of this material in the glomerular capillaries (by immunofluorescence microscopy), documents the participation of this material in immune complex glomerulonephritis. Renal diseases associated with normal and low C3 are enumerated in Table 3-2.

Recent studies indicate that other complement components are also involved; in poststreptococcal glomerulonephritis, components 2 (C2) and 4 (C4) of the serum complement may be transiently depressed but complement component 1 (C1) remains unaltered. In contrast, in lupus glomerulonephritis all the components of the serum complement have been found to be reduced; a low serum C4 value (N = 14–51 mg/100 ml) has been observed prior to a low serum C3 value. A combination of the serum values of total hemolytic complement (CH_{50}) or the components of complement along with the serum titers of anti-DNA antibody constitutes the most valuable guide in the assessment of the therapeutic response in lupus nephritis.

In mesangioproliferative glomerulonephritis, C3 component is markedly reduced; in addition C1 and C4 are also reduced, but it is not yet known whether C2 is involved in this disease. Low serum C3 is accompanied by elevation of serum C3 neF (nephritic factor). The cause for marked reduction of C3 in this

Table 3-2
Serum C3 Values in Renal Diseases

Normal value	Low value
Anaphylactoid nephritis	Chronic hypocomplementemic nephritis
Benign recurrent hematuria	Poststreptococcal glomerulonephritis
Chronic proliferative glomerulonephritis	Serum sickness
Hemolytic–uremic syndrome	Subacute bacterial endocarditis
Hemorrhagic cystitis	Active systemic lupus erythematosus
Hereditary nephritis	
Idiopathic nephrotic syndrome (lipoid nephrosis)	
Interstitial nephritis	
Membranous glomerulonephritis (amyloid, diabetes, renal vein obstruction)	
Orthostatic proteinuria	

Source: Reproduced by the kind permission of Dr. S. P. Gotoff of Michael Reese Medical Center, Chicago, and *Medical Times*.

Table 3-3
Severity of Low Serum C3 Values and Glomerular Deposition of C3 (Immunofluorescence Microscopy) in Different Types of Glomerulonephritis

Glomerulonephritis	C3 hypocomplementemia	Glomerular C3 deposition
Poststreptococcal	+++	+
Lupus	++	+
Mesangioproliferative	+++	+++
Bacterial endocarditis	++	+
Secondary syphilis	0	+
Sclerosing (focal glomerular sclerosis)	0	+
Membranous	0	+
Rapidly progressive	0	0 or +

Source: A large portion of this table is reproduced by the kind permission of Dr. Clark D. West of Cincinnati Children's Medical Center (excerpted from West *et al.*, 1973).

glomerulonephritis is unknown, but it appears to be due to an excessive breakdown of C3 by properdin–zymosan complex, which leads to low serum C3 levels. Serum properdin level is low also in poststreptococcal glomerulonephritis. Table 3-3 shows typical levels for serum C3 and glomerular deposition of C3 in different types of glomerulonephritis. The severity of reduction of all complement components in three major types of immune complex glomerulonephritis is shown in Table 3-4.

Serology of Syphilis

Among the reagin tests, a flocculation reaction, e.g., Venereal Disease Research Laboratory (VDRL), is ordered as a screening test for syphilis in every patient admitted to the hospital. If the VDRL is reactive, an antibody test, e.g., fluorescent treponemal antibody (FTA-ABS), should be obtained in order to

Table 3-4
Severity of Reduction[a] of the Complement Components in Various Types of Immune Complex Glomerulonephritis

Type	C1	C2	C3	C4
Poststreptococcal	0	++	+++	++
Lupus nephritis	++	+++	++	+++
Mesangioproliferative	+	?	+++	+
Glomerulonephritis of chronic bacteremia (e.g., ventriculoatrial shunt infection)	+++	++	++	++

[a] 0, None; +, mild; ++, moderate; +++, severe.
Source: This table has been kindly updated by Dr. Clark D. West of Cincinnati Children's Medical Center, Cincinnati, Ohio (personal communication).

eliminate false positive reaction. Reactive VDRL may occur in lupus glomerulonephritis and glomerulonephritis associated with acute or chronic systemic infectious processes. Since the glomerular changes in syphilitic glomerulonephritis resemble those in lupus membranous glomerulonephritis or idiopathic membranous glomerulonephritis, the laboratory tests may be helpful in differentiating these conditions (Table 3-5).

Erythrocyte Sedimentation Rate

This simple, traditional, bedside test has been found to be helpful in judging the therapeutic response of the nephrotic syndrome. It returns rapidly to normal or near normal when the nephrotic syndrome undergoes remission and stays normal as long as the remission lasts.

Erythrocyte sedimentation rate (ESR) remains elevated when the nephrotic syndrome undergoes an incomplete remission or fails to go into remission. This test then contributes at least some knowledge as to whether the pathology of the nephrotic syndrome is reversible or irreversible. In general, lipoid nephrosis in children or adults is reversible with corticosteroid therapy, whereas a glomerulonephritis which more often causes the nephrotic syndrome in adults is poorly responsive to corticosteroid therapy. In one study of asymptomatic proteinuria and hematuria, elevation of the ESR, along with abnormal quantity of urinary protein, has aided assessment of renal parenchymal disease. These values are normal in orthostatic proteinuria and persistent proteinuria with normal renal histology (including electron microscopy).

Table 3-5
Helpful Serological Tests to Distinguish the Causes of the Membranous Glomerulonephritides

Laboratory tests	Syphilitic GN	Lupus membranous GN	Idiopathic membranous GN
VDRL	Reactive (titer greater than 1:32)	Reactive (titer less than 1:32)	Nonreactive
FTA-ABS (1:5 dilution)	Invariably positive and specific	Negative	Negative
ANA	Negative	Positive (95%)	Negative
Serum complement or components of complement	Normal	Low	Normal
Serum IgM level	High	Normal	Low
Therapeutic test by administration of penicillin	Remission of nephrotic syndrome but effect on histopathology unknown	Unaffected	Unaffected

Blood Smear for Malaria Parasites

Acute glomerulonephritis due to malarial infection appears to be very rare except in African countries. However, the possibility of malarial glomerulonephritis cannot be totally dismissed since increasing worldwide travel facilitates carrier infection from tropical countries in which malaria is endemic to the countries of temperate zones in which malaria is rare. Thick and thin blood films should be examined for malaria parasites, and malaria antibody titers should be obtained if there is a history of possible exposure. It would be extremely difficult to establish a diagnosis of malarial glomerulonephritis based on malarial antibody titers, especially in a traveler from Africa, Southeast Asia, or in countries with endemics of malaria since many asymptomatic individuals from these countries may have high titers of malarial antibody. A diagnosis of malarial glomerulonephritis could be established by exclusion of other causes of glomerulonephritis and consistently high or rising titers of malarial antibody.

Blood Cultures

Blood should be collected for both aerobic and anaerobic cultures before administration of antibiotic to the following types of patients presenting with features of glomerulonephritis: (1) heroin addicts, (2) patients with organic heart murmur, (3) patients with chronic abscess(es), (4) patients with ventriculoatrial (V-A) shunt infection, and (5) patients with clinical evidence of bacterial endocarditis.

Electrophoresis of Serum and Urinary Proteins

Paper Electrophoresis

The following findings may provide some clue to the etiology of glomerulonephritis:

1. Polyclonal rise of gamma globulin with remaining components being normal–nonspecific.
2. Monoclonal spike of gamma globulin with remaining components being normal suggests multiple myeloma, Waldenström's macroglobulinemia, or a rare type of gammopathy. These conditions may become complicated, although rarely, by immune complex glomerulonephritis, but are more often associated with amyloidosis and nephrotic syndrome than with glomerulonephritis.
3. Elevation of $alpha_2$ globulin alone is found in patients with nephrotic syndrome. Bardana and colleagues (1970) have found elevated levels of serum $alpha_2$ globulins in nephrotic syndrome and also in transplant rejection. They are in agreement with the author of this book that clinical improvement in nephrotic syndrome correlates with a decrease of serum $alpha_2$ globulin. Riggio and associates (1968) noted a relationship between rejection of renal grafts and elevation

of serum alpha$_2$ globulin. In the series of studies by these authors, response to immunosuppressive therapy was universally followed by a reduction of this protein fraction in the serum. Therefore, this simple inexpensive test may assist the clinician to estimate the prognosis of nephrotic syndrome and kidney transplant. The test offers no advantage in the management of patients with acute glomerulonephritis.

Immunoelectrophoresis of Serum Proteins

Mild elevations of IgG (N = 564–1765 mg/100 ml) and IgM (N = 53–375 mg/100 ml) in SLE and secondary syphilis, respectively, are not uncommon. Marked elevations of IgG and IgM are found in multiple myeloma and Waldenström's macroglobulinemia, respectively. Serum IgA (N = 85–385 mg/100 ml) level has been found to be elevated in patients with IgA-IgG nephritis (Berger's disease).

In one series of IgE-mediated glomerulonephritis (immunopathology study) comprising 74 patients, serum IgE levels were normal in 85% of patients and elevated in 15% of patients. A third of these 15% revealed glomerular deposits of IgE. Thus, this study reveals no correlation between serum levels of IgE and deposits of IgE in glomeruli.

In general, there is no striking change in the patterns of ordinary electrophoresis or immunoelectrophoresis of serum proteins in glomerulonephritis except in gammopathy, as mentioned earlier. The relationships between the elevated serum IgA level and IgA-IgG nephropathy are difficult to establish. If the IgA nephropathy is primarily due to deposition of IgA in the glomeruli, it is difficult to understand why IgA remains elevated in the serum. This is further complicated by the example of mesangioproliferative glomerulonephritis which reveals heavy deposition of C3 and is often accompanied by persistent C3 hypocomplementemia.

Immunoelectrophoresis of Urinary Proteins

Positive tests for Bence Jones protein or paraproteins in the presence of normal serum immunoglobulins may account for unexplained proteinuria or decreased renal function due to involvement of the kidney by gammopathy.

Serum Cryoglobulins

Cryoglobulins are abnormal serum proteins or protein complexes that undergo reversible precipitation at low temperatures. Two general types are conveniently recognized. The relatively uncommon pure or monoclonal variety consists of a single protein component and occurs typically in multiple myeloma, Waldenström's macroglobulinemia, certain lymphomas, and essential cryoglobulinemia. Of the cryoglobulins, about 20% are of the monoclonal type. A homogeneous cryoprotein structurally related to normal immunoglobulins is present, often in high concentration. The more common category of mixed or multicom-

ponent cryoglobulins appropriately considered as cryoprecipitates results from the interaction of two immunoglobulins, neither of which exhibits cryoprecipitability alone. These complexes, which are often present in relatively low concentration, have attracted increasing attention since the discovery in 1963 that a cryoprecipitate contains IgG and IgM.

Mixed cryoglobulins are freqeuntly found in SLE. The relationship is most striking when the disease is active, renal lesions appear, and serum complement is low. The cryoprecipitates invariably include IgG, IgM, macroglobulin, and the Clq component of complement, which is apparently essential for cryoprecipitation. Some may contain the C3 and C4 components of complement as well.

Coagulation Studies

A thrombotic feature characterized by aggregates of platelets and fibrin inside glomerular capillaries, small arterioles, and peritubular capillaries is not an uncommon finding in glomerulonephritis in particular, in thrombotic thrombocytopenic purpura, and in hemolytic uremic syndrome. Despite clot formation in the kidney, coagulation mechanism remains intact. When clots form in the kidney as a part of disseminated intravascular coagulation (DIC), the partial thromboplastin time (PTT) is prolonged and plasma fibrinogen is low.

Radiological Examination

Radiological studies of the kidneys are of little value in the assessment of either the etiology or activity of glomerulonephritis. A venogram of the inferior vena cava or renal veins may be necessary to exclude occlusion of the renal vein by intraluminal thrombus or external compression of the renal vein as etiological factors in membranous glomerulonephritis. Although controversies exist, most workers tend to believe that renal vein thrombosis is secondary to membranous glomerulonephritis. It has been proposed by a group of investigators that venography may be helpful in cases of proteinuria, with or without edema, if any abnormal findings are encountered. These findings include (1) collateral veins in the flanks with upward blood flow; (2) unexplained edema of the legs associated with mild proteinuria; (3) occurrence of pulmonary embolism in which the source of embolus is not clinically evident; (4) unexplained proteinuria in patients known to be suffering from malignant disease (this condition raises the suspicion of renal vein obstruction, which may be due to direct pressure of the growth on the upper part of the inferior vena cava or be secondary to venous thrombosis); and (5) varicocele of the left testis (left testicular vein drains into the left renal vein).

Note of Caution: It must be remembered that renal vein thrombosis is a strong consideration in nephrotic syndrome occurring for the first time in elderly persons. This is especially important in view of the high incidence of malignant diseases which may potentiate thrombotic process at old age. Renogram and renal scan, using radioisotope materials, ultrasound of the kidneys, and even

renal arteriographic studies evidently have no definite value in the assessment of the etiology of glomerulonephritis. A routine chest film may show a mass lesion in the lung or mediastinum; the mass lesion may be carcinoma, which is increasingly reported to be associated with membranous glomerulonephritis.

Protein Clearance Studies

The protein clearance can be determined by applying the equation

$$C = UV/P$$

where C is the clearance, U is the concentration of protein per 100 ml of urine, V is the volume of urine per minute, and P is the concentration of protein per 100 ml of plasma.

Determination of the Clearance

The clearance can be determined by measuring differential proteins (albumin and globulins) in the urine by either chemical or electrophoretic methods, preferably the latter. Thus, determinations of the amounts of albumin and globulins in the urine would allow one to calculate the clearances of these proteins. A more definitive method is to compare the excretion of albumin with that of a fraction of immunoglobulin (e.g., IgG) by using the equation

$$\frac{C_{IgG}}{C_{Alb}} = \frac{(U_{IgG} \times V)/P_{IgG}}{(U_{Alb} \times V)/P_{Alb}}$$

The ratio C_{IgG}/C_{Alb} is called selective permeability. If this unknown value is multiplied by 100, the value can be expressed as a percentage.

Interpretation of Clearances

The clearance of a protein of known molecular weight provides presumptive evidence of the magnitude of injury to the glomerular basement membrane (GBM). If albumin loss constitutes the bulk of proteinuria, then, with the low molecular weight of albumin (68,000), the ratio becomes very low (less than 15%). This is called highly selective proteinuria, which is compatible with lipoid nephrosis. On the contrary, a greater loss of globulin (e.g., IgG), with its higher molecular weight (250,000), pushes the ratio very high (greater than 30%). This is called poorly selective or unselective proteinuria, which is more consistent with such diseases as membranous or mesangioproliferative glomerulonephritis.

Thus, clearance studies can predict two extremes in glomerular diseases, no damage to the GBM on one end (i.e., lipoid nephrosis), and severe damage to the GBM on the other (i.e., membranous glomerulonephritis). It is often difficult to evaluate many glomerular lesions which fall between these two extremes; nevertheless, it is a reasonable test to distinguish between reversible and irreversible glomerular disease. Notwithstanding the recognized value of this test, there are limitations—for example, proliferative glomerulonephritis—which may

be associated with extensive damage to the GBM and yet be found to have selective proteinuria.

Fibrin Split Products in Serum and Urine

Marked elevations of fibrin split products (FSP) may be found in a variety of cases of proliferative glomerulonephritis including lupus glomerulonephritis (Fig. 3-2). The quantitative assessment of FSP is of little or no aid in determining the etiology of glomerulonephritis. However, FSP in serum and urine are found to be elevated in most cases of proliferative glomerulonephritis (Fig. 3-2). There appears to be a good correlation between the amount of urinary FSP and the degree and distribution of intrarenal fibrin assessed by electron and immunofluorescence microscopy. The serial studies of urinary FSP have been found to be valuable in the assessment of the activity of the acute pathological process. In

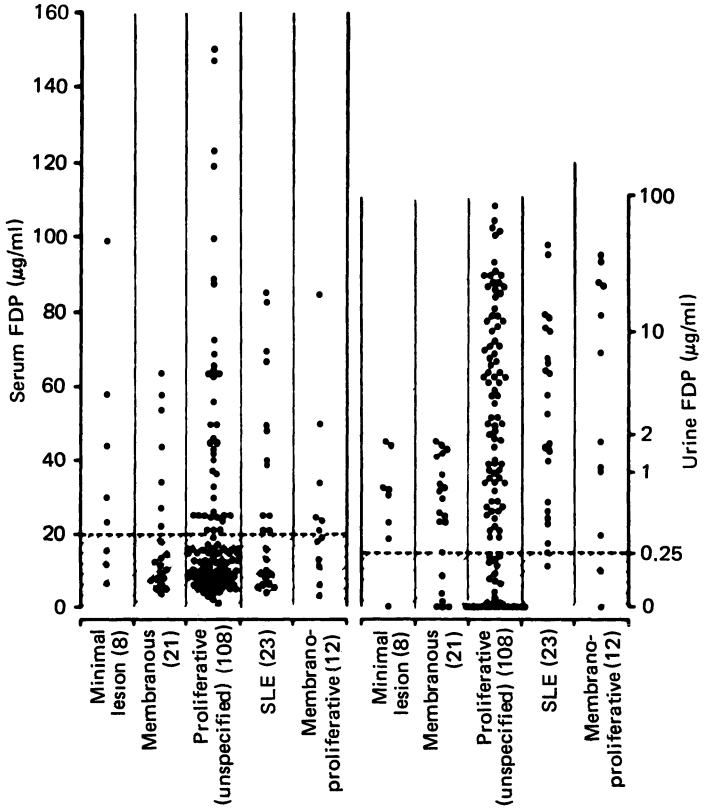

Fig. 3-2. Maximum concentrations of serum and urine fibrin degradation products (FDP, equivalent to FSP) in different types of glomerular diseases. The dashed horitzontal lines across the columns indicate normal ranges. The elevation of FDP in both serum and urine is most marked in proliferative glomerulonephritis (third column in serum and urine). Reproduced by the kind permission of Mary K. MacDonald, M.D., University of Edinburgh, Scotland, and Editor of the *British Medical Journal.*

particular, this test seems to be a valuable index in determining the effectiveness of therapy in glomerulonephritis. Thus, urinary FSP markedly decrease or disappear with arrest of the active pathological process either spontaneously or after therapy.

Viral Antibody Titers

There is sufficient evidence for viral-induced glomerulonephritis in animals and yet there is no direct evidence to implicate virus in the pathogenesis of glomerulonephritis in the human. Acute glomerulonephritis preceded by nonstreptococcal pharyngitis has always been suspected but never proven to be caused by viral infection. Therefore, paired viral antibody titers should be obtained at an interval of 2 weeks. Rising viral antibody titers in a patient with glomerulonephritis, with completely negative studies for bacterial infection or systemic illnesses, will constitute conceptual evidence for viral-induced glomerulonephritis.

SUMMARY

1. Among the various laboratory tests ordered for the diagnostic workup of glomerulonephritis, only a few are useful in establishing the etiology of glomerulonephritis. These are streptococcal antibody titers and antinuclear antibody titers; rising titers or single markedly elevated titers accompanied by immune complex glomerulonephritis provide strong evidence for streptococcal infection or SLE, respectively, as the underlying etiology.

2. Most of the laboratory tests described in this chapter are of value only in the assessment of activity rather than in the diagnosis of the pathological processes. Among these tests, the 24-hr quantitative proteinuria and measurements of complement components constitute the more reliable indices of the activity of glomerulonephritis.

3. Complete disappearance of proteinuria (N = 100–150 mg/24 hr) and repeatedly normal urinalysis indicate reversible histological lesion.

4. Persistent or recurrent proteinuria suggests an active or progressive lesion, regardless of the etiology.

5. A combination of urinary sediment and ESR also aids in the evaluation of the activity of the pathological process; persistently abnormal urinary sediments, along with persistently elevated ESR, are supportive of an active or ongoing disease process. The author would like to emphasize that examination of the urinary sediment and ESR should be a "must" in every clinic visit of patients with glomerular disease until they recover or culminate in end-stage disease.

6. In SLE, there is a close relationship among native anti-DNA antibodies, serum complement (C3) levels, and renal disease. An active stage of the renal disease is accompanied by high anti-DNA antibody titers and low serum C3

levels. During remission of lupus GN, spontaneous or induced by treatment, ANA and anti-DNA titers decline but seldom become negative. The ESR falls, proteinuria is reduced in amount, LE cells often disappear, and serum C3 returns to normal or near normal values.

7. There is enough evidence to indicate excellent correlation between serum complement components and activity of the disease processes. Thus, in acute immune complex glomerulonephritis (poststreptococcal or glomerulonephritis due to bacterial endocarditis), serum C3 component remains low until 2 to 6 weeks from the onset of the disease; it returns to normal during recovery (rarely up to 12 weeks after onset). In lupus GN, lowering of serum C4 is found earlier than the lowering of serum C3. During remission (spontaneous or induced by therapy) both components rise to normal or near-normal values. In mesangioproliferative glomerulonephritis, especially in children, serum C3 tends to remain persistently low.

8. In general, blood urea nitrogen and serum creatinine correlate poorly with pathological activity. In some instances, however, modest but progressive increase may be associated with smoldering glomerular disease such as focal glomerular sclerosis (sclerosing glomerulonephritis).

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4 Glomerulonephritis: Pathogenesis and Classification

DEFINITION

Glomerulonephritis is defined as a clinicopathological condition which characteristically involves both kidneys or only one kidney when the other kidney is congenitally absent or has been removed due to trauma, tumor, or abscess. It is characterized by the deposition of immunoglobulins (or antibody) in the glomeruli, sometimes in the tubules, and, rarely, in the interstitium and arterioles. These immunoglobulins are observed as deposits by electron and immunofluorescence microscopy. Frequently, neutrophilic leukocytes and complement components are also identified in the lesions. Other forms of glomerulonephritis, in which immunoglobulin and complement deposition are absent, also occur.

PATHOGENESIS

Since the introduction of electron microscopy into the study of renal tissue, it has become possible to observe fine changes in all the component parts of the kidney in glomerulonephritis. Immunofluorescence microscopy using fluorescein–isothiocyanate conjugated heterologous antisera against various human immunoglobulins and complement components, in conjunction with electron microscopy, has clarified several mechanisms that could lead to glomerulonephritis. Although in all instances an antigen (exogenous or endogenous) is believed to be involved in the formation of antigen–antibody complex or immune complex and in the pathogenesis of immune complex glomerulonephritis, in only a few studies has the antigen been unequivocally proven to be present.

Two broad types of antibody mechanisms have been implicated in the pathogenesis of glomerulonephritis: (1) anti-glomerular basement membrane antibody (anti-GBM type) and (2) antigen–antibody complex or immune complex. The characteristic renal morphological abnormalities in the two types of human glomerulonephritis resemble those in experimentally induced glomerulonephritis in animals. Although these two types constitute the vast majority of glomerulonephritides, a small percentage of glomerulonephritis is associated with poorly defined mechanisms and less specific or nonspecific glomerular abnormalities.

Anti-Glomerular Basement Membrane Antibody Glomerulonephritis

Anti-glomerular basement membrane antibody glomerulonephritis (anti-GBM/GN) which is clinically known as rapidly progressive glomerulonephritis, constitutes about 5% of the total number of glomerulonephritis cases. In this type of glomerulonephritis autologous glomerular basement membrane (GBM) apparently acts as an antigen and incites production of antibody against GBM. This antibody then binds with the GBM and through this interaction initiates the glomerular injury, with the consequent clinical picture of glomerulonephritis. The experimental model of Stebley (1962) is very useful in understanding the pathogenesis of anti-GBM/GN or rapidly progressive glomerulonephritis. Stebley reproduced the glomerular changes of rapidly progressive glomerulonephritis by injecting human GBM and Freund's complete adjuvant into monkeys and sheep. Further, he and his colleagues postulated that this type of glomerulonephritis occurs via anti-GBM antibody. This hypothesis became evident when Lerner and Dixon (1966) demonstrated that this disease could be transferred to normal sheep with IgG from the serum of afflicted animals as well as by renal elutes. This observation establishes the presence of a true autoimmune disease characterized by the formation of antibodies to the animal's own glomerular basement membrane and to that of a homologous animal. The glomerular changes in this model are characterized by crescent formation, necrosis, and collapse of glomerular capillaries. In addition, these glomerular changes are often associated with severe interstitial cellular infiltration, fibrosis, and tubular atrophy. The presence of a continuous linear electron-dense deposit with destructive GBM changes and linear fluorescence against IgG and C3 found in the GBM by electron microscopy and immunofluorescence microscopy, respectively, provides conceptual evidence for the anti-GBM antibody glomerulonephritis.

In general, the model of nephrotoxic serum nephritis or Masugi's (1933) nephritis has been considered during the past several decades as an important model for rapidly progressive glomerulonephritis. Masugi's model appears to be similar to that of Stebley, but some fundamental differences do exist, as shown in Table 4-1.

The similarity of the light microscopic appearance of the kidneys in these

Table 4-1
Differences between Masugi's and Stebley's Nephritides

	Masugi (1933)	Stebley (1962)
Material used	Rat antisera against rabbit kidney	Human GBM and Freund's complete adjuvant
Immunological nature of the material	Heterologous antibody	Heterologous antigen
Autoimmunity	No	Yes
Mechanism of glomerular injury	Unknown	Anti-GBM antibody deposition
Morbid anatomy	Crescentic glomerulonephritis	Crescentic glomerulonephritis with disruptive changes in GBM

two experimental models and their resemblance to the renal pathology in Goodpasture's syndrome (to be classified later), or rapidly progressive glomerulonephritis, suggest a similar pathogenetic mechanism for rapidly progressive glomerulonephritis. Since linear fluorescence against IgG and C3 has been observed in the GBM in such conditions as diabetes mellitus and systemic lupus erythematosus (SLE), some reservations must remain in considering linear fluorescence as the sole criterion in defining the mechanism of anti-GBM antibody glomerulonephritis.

The mechanisms by which GBM incites antibodies against the autologous component and establishes the conditions for glomerulonephritis are still unknown. Two possibilities have been suggested:

1. Production of antibodies by alteration of the endogenous GBM. The finding of GBM antigen(s) in the urine of normal animals and humans, and the nephritogenicity of this antigen when it is injected into the animal from which it was obtained, suggest that such urinary antigens might be the immunogens in human nephritis.
2. Stimulation of the GBM by an exogenous agent or agents, e.g., streptococci or a virus, for which there is still no supportive evidence.

Immune Complex Glomerulonephritis

This type of glomerulonephritis accounts for the vast majority (75–80%) of glomerulonephritis cases encountered in clinical practice. The classical immune complex is composed of an antigen, one or more antibodies, and a complement component. The immunoglobulins (antibodies) and complement components have been demonstrated in most or all instances, but in only a few of these has an antigen been unequivocally demonstrated. The antigens that have been implicated and are believed to be involved in the formation of immune complex and immune complex glomerulonephritis in humans are shown in Table 4-2.

The experiments of acute "one-shot" serum sickness nephritis in rabbits conform to acute immune complex glomerulonephritis in humans. If rabbits are

Table 4-2
The Antigens Implicated in the Pathogenesis of Different Types of Glomerulonephritis

Antigen involved	Diseases	Evidence
DNA	Lupus glomerulonephritis	Almost unequivocal
Hemolytic *Streptococcus*	Poststreptococcal glomerulonephritis	Almost unequivocal
Streptococcus, Staphylococcus aureus	Glomerulonephritis in bacterial endocarditis	Equivocal
Staphylococcus	Glomerulonephritis in ventriculoatrial shunt	Doubtful
Plasmodium malariae	Malarial nephritis	Almost unequivocal
Treponema pallidum	Glomerulonephritis in secondary syphilis	Equivocal
HAA	"Diffuse proliferative glomerulonephritis"	Unequivocal
Tumor antigen	Membranous glomerulonephritis	Doubtful
Penicillin	"Diffuse proliferative glomerulonephritis"	Equivocal
Penicillamine	Membranous glomerulonephritis	Equivocal

given one intravenous injection of heterologous serum, such as bovine serum albumin, the animals develop glomerulonephritis in 10 to 14 days. Histologically, this type of glomerulonephritis is characterized by generalized and diffuse proliferation of endothelial and mesangial cells of the glomeruli, with variable exudation of polymorphonuclear leukocytes inside the glomerular capillary lumina. The presence of discrete but intermittent electron-dense deposits in the different aspects of the GBM and in the mesangium, as demonstrated by electron microscopy, and of brilliant discontinuous or granular staining with antisera against IgG and/or C3, observed along glomerular capillaries and mesangium by immunofluorescence microscopy, constitutes strong evidence of glomerular injury by immune complex mechanism.

A major advance in our potential understanding of human glomerulonephritis has been obtained by the study of spontaneous glomerulonephritis in animals. In all instances in which this phenomenon has been studied, it has been shown to be an immune complex disease, and in all cases the antigenic component has been found to be a virus. In mice chronically infected with the virus of lymphocytic choriomeningitis and in minks with Aleutian disease, the immune complex is composed of the infectious agent plus IgG and C3. In these instances, the virus apparently persists in the circulation throughout the life of the animal, thereby providing the antigen for immune complex formation. Of particular interest is the immune complex nephritis which occurs in the hybrid of black and white strains of New Zealand mice (NZB × W). This disease closely resembles human SLE. The animals have antinuclear antibodies, a Coombs-test-positive hemolytic anemia, and the deposition of nuclear antigen–antinuclear antibody–C3 complexes in the glomeruli. The fact that in human lupus nephritis, complexes of DNA and anti-

DNA antibodies have been clearly demonstrated in the kidney, the fact that DNA and RNA are integral parts of viral structure, and the ubiquitous presence of the viruses in the environment make these agents highly suspect in the role of human immune complex disease.

There are two questions which should be answered in considering the direct role of these immune complexes in the pathogenesis of glomerulonephritis: Why does immune complex deposition occur in the kidney in the first place? Is there any direct evidence that the immune complex alone produces glomerulonephritis?

There is a general consensus of opinion that immune complexes, when formed in excessive amounts, become trapped in the glomerular capillaries during filtration. Present experimental and clinical data strongly support this contention. The concomitant appearance of glomerular hypercellularity and outpouring of polymorphonuclear leukocytes (PMN), along with the electron-dense deposits or the granular fluorescence at the onset of the illness, and the disappearance of most of the features after 10 to 12 weeks constitute strong circumstantial evidence of the interaction of these immune complexes in the evolution of the clinicopathological syndrome of acute glomerulonephritis. This concept is further supported by the fact that intravenous injection of soluble immune complexes produces serum sickness nephritis in mice. Immune complexes have been demonstrated to cause adherence of neutrophils. The phagocytosis of the complex is accompanied by the release of a wide variety of chemotactic substances, including cathepsins and permeability factors, all of which add to the tissue damage occurring at the site of the deposits.

OTHER MECHANISMS OF GLOMERULONEPHRITIS

IgA-IgG Nephropathy (Berger's Disease)

This type of nephropathy is characterized by positive fluorescence, largely for IgA and to a lesser degree for IgG, mainly in the glomerular mesangium on immunofluorescence microscopy of the glomeruli. Histologically, this condition appears as focal proliferative glomerulonephritis. The antigenic source is unknown, and the causal relationship between mesangial deposits of IgA-IgG and histological finding of focal proliferative glomerulonephritis is unclear.

Lipoid Nephrosis

The pathogenesis of lipoid nephrosis (nil lesion or minimal lesion disease) is completely unknown. There are occasional reports which have suggested IgE deposition as a pathogenetic mechanism in lipoid nephrosis. This notion was based on the observation of positive immunofluorescence for IgE in the glomerular capillaries of some children with nephrotic syndrome. This hypothesis was supported further by the combination of normal histology (light microscopy) and the appearance of lipoid nephrosis (electron microscopy). Additional supportive

evidence for this idea is the higher incidence of allergic disorders (IgE mediated) in patients with lipoid nephrosis and in their family members. The original observations of IgE deposition have not been substantiated by other investigators, however, and the occasional occurrence appears to be one that is not causally related to the disease, but is related to the coexisting allergic disorders in the same patients.

CLASSIFICATION OF GLOMERULONEPHRITIS

In the past, there was no consistent system by which glomerulonephritis was classified. For example, Volhard and Fahr (1914) used the terms acute, subacute, and chronic nephritis, whereas Ellis (1942) used type I and type II nephritis. The obvious reasons for this variability of classification were the limited facilities and a failure to identify definite agent(s) which might produce glomerular inflammation. During the last two decades knowledge of the mechanisms of glomerular inflammation has improved due to easy access to the kidney through the technique of percutaneous biopsy, the availability of fine instruments (electron microscope and immunofluorescence microscope) to study the renal tissue, and a variety of supplemental diagnostic aids to assay serum factors that may be involved in the glomerular inflammatory process. Despite our awareness of the morphological details in glomerulonephritis, and despite the widespread availability of these facilities, the exact nature of the exogenous or endogenous agent(s) inducing human glomerulonephritis has remained largely unconfirmed. Nevertheless, except for definition of the exact inciting factors, glomerulonephritis is currently much better understood.

PREVALENT CLASSIFICATION

The prevalent classification is made up of histopathological changes based on predominant abnormalities found in the glomeruli by light microscopy. In early 1960, Allen (1966) introduced the most comprehensive classification, which has since been modified but the original concepts of which remain unchanged. Allen's classification of glomerulonephritis is as follows:

I. Diffuse glomerulonephritis
   A. Acute
      1. Proliferative
      2. Exudative
      3. Necrotizing
      4. Membranous
      5. Lobular
   B. Subacute

C. Chronic
   1. Membranous } Nephrotic syndrome
   2. Lobular }
   3. Sclerosing

II. Focal glomerulonephritis
   A. Segmental
      1. Lupus nephritis
      2. Necrotizing, allergenic (pseudoembolic)
      3. Embolic
   B. Nonsegmental
      1. Proliferative
      2. Exudative
      3. Necrotizing
      4. Membranous

Cameron (1972) has classified glomerulonephritis as follows:

1. Minimal change
2. Membranous nephropathy
3. Focal glomerulosclerosis
4. Proliferative glomerulonephritis
   a. Acute exudative
   b. Mesangial
   c. Epithelial (rapidly progressive glomerulonephritis)
   d. Mesangiocapillary (membranoproliferative, lobular)
   e. Focal
   f. Chronic endothelial (endocapillary)
   g. Advanced sclerosing lesions, unclassifiable

There are some differences which still exist among the histological classifications, but these appear to be matters of expression rather than recognition of different entities. I would like to propose the following classification, which should agree with that of most authors:

A. Diffuse glomerulonephritis
   1. Proliferative
   2. Membranous
   3. Mesangiocapillary (mesangioproliferative, membranoproliferative, lobular)
B. Focal glomerulonephritis
   1. Focal proliferative
   2. Focal membranous
   3. Focal sclerosing

Proliferative glomerulonephritis may be accompanied by (a) predominantly endothelial proliferation, which is called endocapillary proliferative glomeruloneph-

ritis; (b) predominantly extracapillary proliferative change, i.e., proliferation of glomerular and Bowman's epithelial cells (this epithelial proliferation forms crescents which characterize crescentic glomerulonephritis); (c) predominant proliferation of mesangial cells, called mesangioproliferative glomerulonephritis; or (d) a combination of proliferation of endothelial, epithelial, and mesangial cells. Both focal proliferative and focal membranous glomerulonephritis are found in lupus glomerulonephritis. Focal proliferative glomerulonephritis alone may be found in bacterial endocarditis or the Henoch–Schonlein syndrome. Focal sclerosing glomerulonephritis is also called focal and segmental glomerular sclerosis.

MERITS AND PITFALLS OF ALL CLASSIFICATIONS OF GLOMERULONEPHRITIS

Although the classifications of nephritis introduced by Volhard and Fahr and by Ellis are now obsolete, the value of these early attempts at classification cannot be denied. The clinical presentations in acute or type I nephritis resemble those observed in the majority of cases of diffuse proliferative glomerulonephritis. Thus, the old acute or type I nephritis may be used fairly well as a synonym for diffuse proliferative glomerulonephritis.

However, in current practice, the practical value of the older classifications has been jeopardized when inconsistent relationships between the anatomic pathology and the clinical presentations are recognized. An example is the morphology of the subacute nephritis of Volhard and Fahr (florid crescent formation). The association of the nephrotic syndrome with subacute nephritis (Volhard and Fahr) is far from the rapid and irreversible renal failure almost always found to be associated with severe crescentic glomerulonephritis or rapidly progressive glomerulonephritis. In addition, a wide spectrum of glomerular lesions which are found to be associated with the nephrotic syndrome bear slight or no similarity to the histology of type II nephritis. Thus, a knowledge of the fine pathology of the kidney and the usage of current histological classifications have been helpful in planning appropriate management and predicting the course of the illness, in spite of the limitations of those classifications.

Allen's classification is essentially up to date, but is somewhat repetitive. There is no rationale for subdividing diffuse glomerulonephritis into separate proliferative and exudative lesions. In most instances, proliferative glomerulonephritis is accompanied by a variable degree of exudation.

PERSPECTIVES OF GLOMERULONEPHRITIS

In all probability, glomerulonephritis would best be classified according to the underlying pathogenetic mechanisms, a proposal that is in conformity with the studies of Cameron (1972) and McCluskey (1973). The mechanisms which are generally accepted are (1) immune complex glomerulonephritis and (2) anti-GBM

glomerulonephritis. A less accepted mechanism is (3) IgA-IgG nephritis (Berger's disease), and the least accepted mechanism is (4) IgE nephropathy (lipoid nephrosis). Most present-day authorities do not accept the pathogenetic implications of IgE in lipoid nephrosis.

Histologically, anti-GBM glomerulonephritis is a less definitive lesion, since the glomerular cresents that characterize this type of glomerulonephritis are observed also in immune complex glomerulonephritis. Similarly, continuous linear deposits seen by electron microscopy and linear fluorescence against IgG and C3 by immunofluorescence microscopy, considered to be the hallmark of anti-GBM glomerulonephritis, may be found in immune complex glomerulonephritis such as lupus nephritis and in diabetic nephropathy. Therefore, the distinct entity of anti-GBM glomerulonephritis has been the subject of controversy. In addition, light microscopy may reveal crescentic glomerulonephritis, and furthermore electron microscopy and immunofluorescence microscopy demonstrate discrete electron-dense deposits and granular fluorescence, respectively.

On the other hand, immune complex glomerulonephritis appears to be a specific entity, since the microscopy features are consistent with immune complex and a linear deposit or linear fluorescence is seldom demonstrated. Furthermore, in immune complex glomerulonephritis, the location of electron-dense deposits in relation to the glomerular basement membrane, alterations of GBM, and changes in the mesangium may provide some clues with respect to the etiological factors of the disease process. For example, typical "humps" most commonly implicate a poststreptococcal glomerulonephritis, whereas discrete subendothelial deposits are most frequently found in lupus nephritis. This type of knowledge is helpful in planning treatment and in determining the course of the disease in patients with glomerulonephritis. However, the factors of time and expense, and the lack of an adequate number of skilled personnel limit the full application of electron and immunofluorescence microscopy in the study of glomerulonephritis.

CRITICISM OF CURRENT HISTOLOGICAL CLASSIFICATION

Minimal lesion or nil lesion disease (lipoid nephrosis) is still included by many in their classifications of glomerulonephritis, despite the negative evidence for inflammatory reaction or immunological involvement. The prominent glomerular mesangium or slight increases in mesangial matrix and cellularity sometimes found in lipoid nephrosis are not sufficient to include lipoid nephrosis in the category of glomerulonephritis. Michael *et al.* (1973) have characterized nil or minimal lesion (i.e., lipoid nephrosis) as the idiopathic nephrotic syndrome. Perhaps this is not the best term for lipoid nephrosis, but at least it distinguishes lipoid nephrosis from nephrotic syndrome secondary to glomerulonephritis.

The mild glomerular changes reported by light microscopy alone should be accepted with reservation. This pertains especially to mixed proliferative and membranous glomerulonephritis or membranoproliferative glomerulonephritis,

which by electron microscopy appears to be membranous glomerulonephritis in most cases and mesangioproliferative glomerulonephritis in a few cases.

With electron microscopy, three definite types and one less definite type of glomerulonephritis can be demonstrated: (1) proliferative, (2) membranous, (3) mesangioproliferative, and (4) (less definite) sclerosing.

CHRONIC GLOMERULONEPHRITIS

With the advancement of our knowledge in the studies of anatomic pathology of renal diseases, we believe that it is no longer a valid proposition to denote a microscopic picture of hyalinized (solid) glomeruli, atrophic or dilated tubules, and interstitial infiltrates and fibrosis (*burnt-out kidney*) as chronic glomerulonephritis. The term *chronic* means continuing or lasting. Therefore, it would be a logical practice to use the term *chronic* for any type of glomerulonephritis that reveals features of persistent activity or progression. Then, by definition, membranous, mesangioproliferative, or sclerosing glomerulonephritis should be regarded as chronic glomerulonephritis, an idea that is in agreement with that of Allen (see Allen's classification). Endocapillary proliferative glomerulonephritis (e.g., poststreptococcal GN) may fail to undergo complete resolution and manifest features of persistent pathological activity. This entity should also be included in the list of chronic glomerulonephritis.

When light microscopy demonstrates a picture of burnt-out kidney and a preceding histologic picture is unknown or unavailable, it is unwise to assess a primary pathology, e.g., glomerulonephritis, glomerulosclerosis, or chronic interstitial nephritis (chronic pyelonephritis), on the basis of these changes. The findings of relatively severe damage in the individual components, e.g., glomeruli, arterioles, or interstitium, may lead the observer to speculate that the primary disease process might have been glomerulonephritis, nephrosclerosis, or tubulointerstitial disease, respectively. Some investigators consider a proliferative glomerulonephritis as the underlying disease based upon observations of an excessive number of nuclei present in hyalinized glomeruli or in a few surviving glomeruli. Still others have stated that the presence of a thickened basement membrane in the patent capillaries of hyalinized glomeruli suggests membranous glomerulonephritis as the primary disease process. This type of guessing involves great risks and should be avoided. Furthermore, the anatomy of the kidney at this stage of the disease is so distorted that any attempt to obtain more information using electron or immunofluorescence microscopy becomes futile. Therefore, this type of microscopic picture should be described as an "end-stage kidney" and institution of specific drug or other therapy based on such meager information should be abandoned, especially since the disease has, by definition, already reached an irreversible stage.

SUMMARY

1. All glomerulonephritis should ideally be divided into anti-GBM antibody glomerulonephritis and immune complex glomerulonephritis. Of the total number of glomerulonephritides, 75 to 80% are of the immune complex type, whereas 5% or less appear to be caused by anti-GBM antibody. The mechanism is obscure in 15 to 20% of patients with glomerulonephritis.

2. Electron and immunofluorescence microscopy studies are essential to distinguish the different types of glomerulonephritis.

3. Anti-GBM antibody glomerulonephritis appears to be less specific than immune complex glomerulonephritis since linear fluorescence against IgG and C3, which characterizes anti-GBM antibody glomerulonephritis, has been observed in diabetic glomerulosclerosis, lupus nephritis, and membranous glomerulonephritis. In immune complex glomerulonephritis, the immune complex is presumably composed of an antigen and one or more antibodies. Although in all instances an antigen is believed to be present in the immune complex, its presence has been documented in only a few instances. In only a few anti-GBM glomerulonephritis cases are the disruptive GBM changes conceivably due to antibody directed against GBM. In immune complex glomerulonephritis, however, it remains undetermined whether the histological changes are the direct effect of immune complex deposition, or occur indirectly via other mechanisms.

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5 Electron Microscopy of Normal Kidney

INTRODUCTION

For electron microscopy (EM) study of the kidney, the renal tissue must be fixed in 4% glutaraldehyde and postfixed in 1% osmium tetroxide (OsO_4). The details of fixation, embedding, sectioning, and staining methodology are given in Chapter 2. For convenience of study and analysis, the anatomy of the renal parenchyma can be divided into four component parts: glomerulus, tubule, interstitium, and small arterial vessels (small artery, large arteriole, and small arteriole).

Since this book is intended for the clinician, practicing physician, and pathologist, this chapter includes materials obtained from studies of human renal biopsies. It also deals primarily with the broad general aspects of the fine structures in order to fulfill the clinical needs only. It should be stated, however, that renal biopsy cannot be performed in normal subjects and that the autopsy material is suboptimum for electron microscopy study. Therefore, the electron microscopic anatomy is limited mainly to renal biopsy studies which were found to be essentially normal in patients with hematuria or mild proteinuria and only minimally to studies of kidneys from a small number of heart-beating cadaver donors.* Electron microscopy studies of the kidneys from normal dogs or rats are briefly included to show similarities of the normal renal morphology in the different species.

* Their kidneys were harvested for the purpose of transplantation but were subsequently found unsuitable because of low antigen match.

GLOMERULUS (Figs. 5-1 to 5-10)

Electron microscopic study of a glomerulus resolves the following: (1) glomerular basement membrane (GBM); (2) endothelial cell (END); (3) visceral epithelial cell (VEP); (4) epithelial foot processes (FP); (5) mesangium (MES); (6) Bowman's membrane, Bowman's space, and parietal epithelial cell (BWM, BS, and PEP, respectively); and (7) juxtaglomerular apparatus (JGA). A glomerulus has about five arbitrary lobes and each lobe has eight to ten capillaries. Therefore, a single glomerulus has 40 to 50 capillaries. Each glomerular capillary has two parts: peripheral and centrilobular. The peripheral portion of the capillary

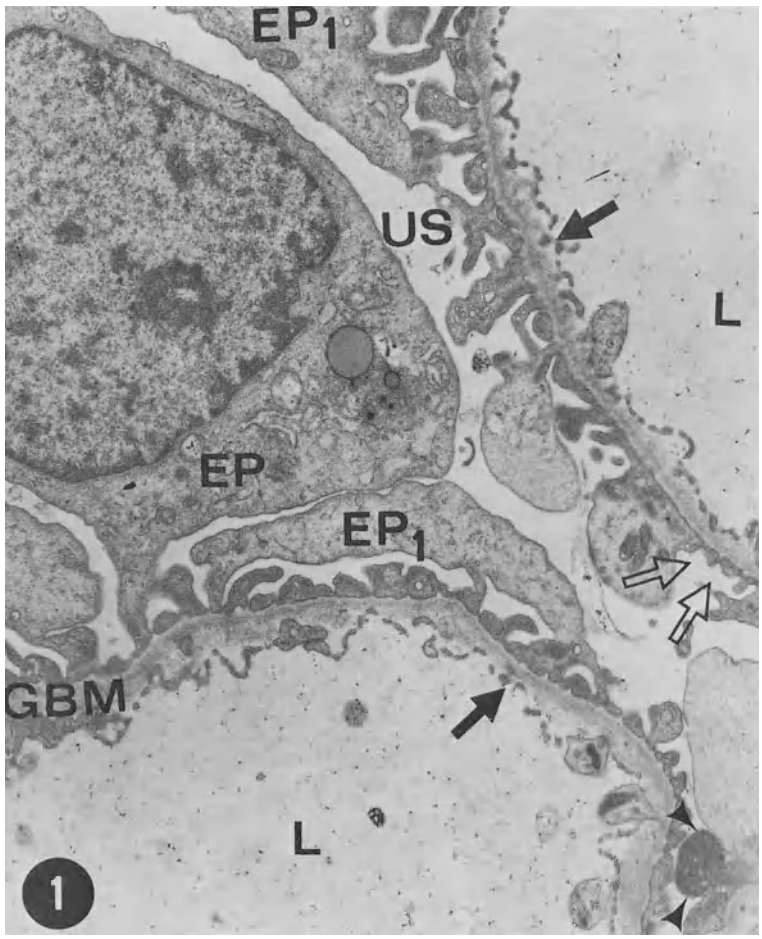

Fig. 5-1. Two glomerular capillaries. Endothelial cytoplasm appears as fenestrae (solid arrows). Note large nucleus and many rough-surfaced endoplasmic reticula in the epithelial cell (EP). Some epithelial cells (EP_1) are devoid of nuclei but attached to glomerular basement membrane (GBM) by more than one foot process. Discrete foot processes (open arrows) and myofilament (arrowheads) are shown, as are lumen of the glomerular capillary (L) and urinary space (US) (UA + LC, ×16,000).

loop has one GBM, one or two endothelial cells, and one or two epithelial cells (Figs. 5-1 to 5-5). The GBM is a homogeneous solid layer with the appearance of sandpaper; it is interposed between the epithelial cell foot processes and endothelial cells. Many investigators have described GBM as consisting of three layers, a central expanded portion called lamina densa and two layers of thin membranes called lamina rara interna and lamina rara externa on the inner and outer aspects of the lamina densa, respectively. Careful observation reveals the absence of a complete membrane either on the endothelial or the epithelial aspect of GBM. Instead, short segments of thin membrane called *slit membranes* are seen between foot processes on the epithelial aspect (Fig. 5-4). This membrane, which is 50 Å thick, does not appear to be PAS positive and is not observed in PAMS-stained sections (Fig. 5-5). Interrupted membranes called *endothelial fenestrae* can be found on the endothelial aspect of the lamina densa (Figs. 5-1 and 5-3). The GBM is silver-positive and shows uniform deposits of silver particles throughout (Fig. 5-5). This silver reaction supports the glycoprotein composition of GBM. The GBM is thicker than the basement membrane of a capillary of the terminal vascular bed and measures 2500 to 3500 Å in humans. This thickness

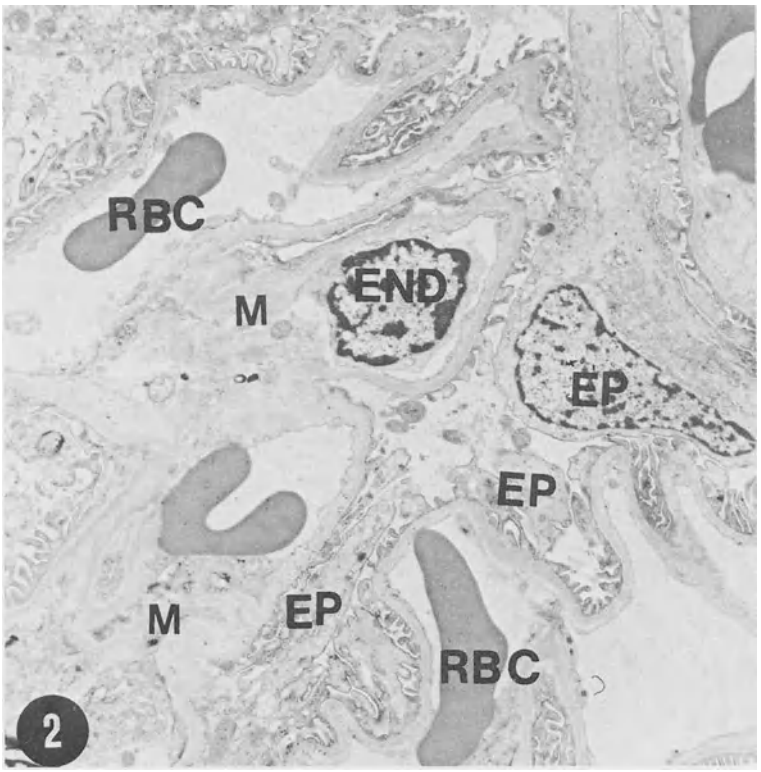

Fig. 5-2. Several normal-appearing glomerular capillaries. Endothelial cell (END), mesangium (M), and epithelial cell (EP) are shown. Normally the capillary loops appear empty or contain one or two red blood cells (RBC) (UA + LC, ×3400).

varies, however; the centrilobular portion is thicker than the peripheral portion (Figs. 5-2 and 5-3).

The GBM generally is smooth on both endothelial and epithelial sides but slight irregularities on the endothelial aspect of the GBM are not uncommon (Figs. 5-1 and 5-2). This type of irregularity has no significance, especially if it occurs in the centrilobular portion of the capillaries (Fig. 5-3). The endothelial irregularities of the centrilobular portions of the capillaries may be an artifact and may often be caused by a tangential cut across the GBM. This becomes certain if clusters of small holes are also found within the GBM.

Endothelial Cells (Figs. 5-2 to 5-5)

A glomerular capillary has one or two endothelial cells which vary in size, shape, and cytoplasmic constituents. In general, there is a single nucleus which also varies in size and shape. The nucleus is usually large and occupies 70 to

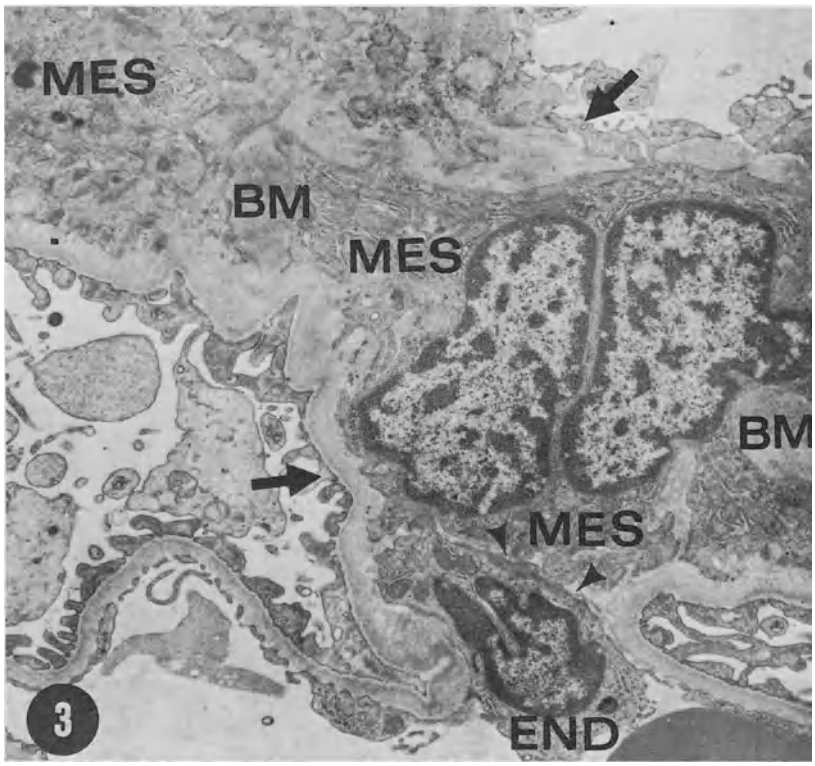

Fig. 5-3. Several glomerular capillaries. In one glomerular capillary, endothelial cell (END) is seen separated from the mesangial cells (MES) by a thin basement membrane-like material (arrowheads). The mesangium is bounded by the centrilobular portions of the glomerular capillaries (arrows) and surrounded by basement membrane-like materials (BM) (UA + LC, ×12,000).

80% of the cell area. In some capillaries there is no hiatus between the endothelial cell and the GBM (Figs. 5-2 to 5-4), whereas in other capillaries endothelial fenestrae intervene, at least in part, between the GBM and the endothelial cell (Fig. 5-6). Cytoplasmic constituents consist of a few rough-surfaced endoplasmic reticulum, a few mitochondria, moderate to large amounts of ribosomes, and Golgi complexes. In addition, one or more vacuoles may be found within the endothelial cell.

Visceral Epithelial Cells (Figs. 5-1, 5-2, 5-4, 5-5)

Each glomerular capillary generally has one epithelial cell, which is the largest of all cells in the glomerular capillaries. This cell has a nucleus and is rich

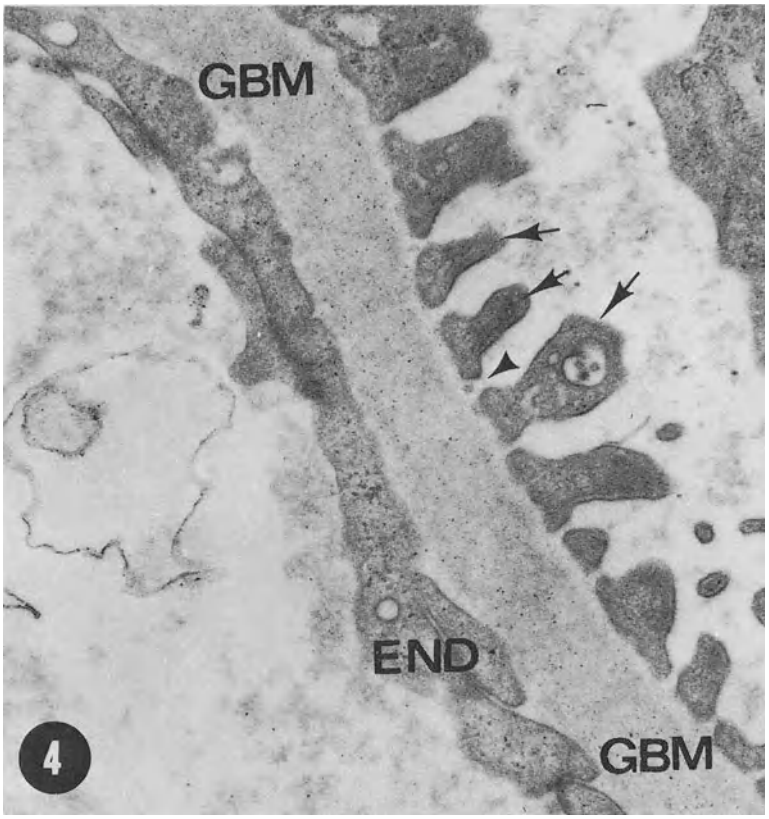

Fig. 5-4. Magnified view of a peripheral portion of a glomerular capillary. Note the glomerular basement membrane (GBM) which has the appearance of a solid bar. Discrete foot processes (arrows), a thin membranelike structure between foot processes (arrowhead), and endothelial cells (END) are shown. The foot processes are surrounded by membranes which will be delineated in subsequent micrographs. No membrane described as lamina rara interna or lamina rara externa on the inner or outer aspect of GBM, respectively, can be documented from this study (UA + LC, ×26,000).

in organelles. The nucleus occupies one-half to three-fourths of the cell (Fig. 5-1). The cytoplasm is enriched with Golgi complexes and endoplasmic reticulum (ER). Some endoplasmic reticula are rough surfaced, some are smooth, and others are dilated, and all three of these characteristics constitute a conspicuous feature of the epithelial cell (Figs. 5-1 and 5-7). Mitochondria are moderate in number (Fig. 5-7). A few vacuoles and a few lipid droplets are frequently observed. The epithelial cell is attached to the GBM by one or more limbs and has the appearance of an umbrella over the GBM (Figs. 5-1, 5-5, and 5-7). One or more limbs of the epithelial cell may extend to the neighboring capillaries (Fig. 5-7). Myofibrils and myofilaments are easily discernible (Fig. 5-1); the similarities of these structures to the contractile elements of other cells suggest the contractile potential of the glomerular epithelial cells. Besides one large epithelial cell, several small epithelial cells are seen attached to GBM by one or more foot processes. These small epithelial cells are devoid of nuclei and have the appearance of small animals crawling over the GBM (Figs. 5-1 and 5-7).

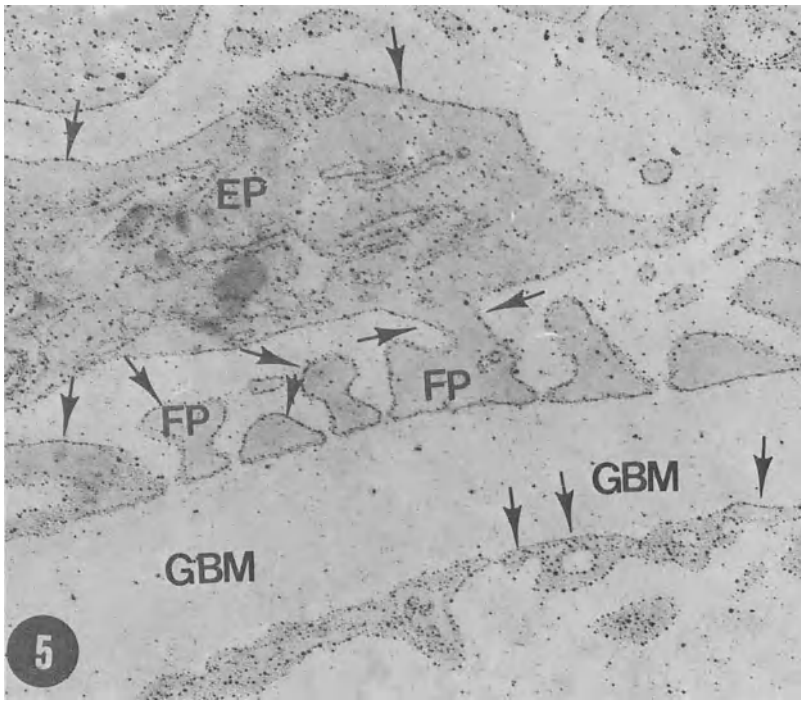

Fig. 5-5. Definition of membrane structures by the silver staining technique. Note membranes (arrows) consisting of fine deposits of silver surrounding foot processes (FP), and epithelial cell (EP). Note continuity of EP with FP (pointing arrows), openings between individual foot processes (these openings are regarded as filtration pores), and also the absence of continuous membranes (either lamina rara interna or externa). In fact, it is the cellular membranes which might have been misinterpreted as lamina rara interna or externa. The glomerular basement membrane (GBM) is not stained as heavily as the cellular membranes. (PAMS, ×28,000).

Foot Processes (Figs. 5-1, 5-4 to 5-7)

The foot processes are the most unique structures of the glomeruli observed by EM study. These structures have been described as cytoplasmic extensions of the epithelial cell, although they do not appear to be that. The continuum of the epithelial cell with a segment of GBM via one to four foot processes is the usual finding (Figs. 5-1, 5-6, and 5-7), but many foot processes intervene in the GBM between the extended portions of the epithelium. These intervening foot processes appear to arise from the epithelial cell of one or more adjacent glomerular capillaries; therefore, a syncytial or interdigitating network of glomerular epithelial cells is conceivable. Scanning electron microscopy has demonstrated a thin network pattern. The foot processes are quite discrete and spaced equidistantly 0.1 to 0.5 μm apart (Figs. 5-4 to 5-6). A very thin membrane called a slit membrane may be found between adjacent foot processes.

The foot processes are surrounded completely by thin membranes which separate them individually and from the GBM. This is best observed in a PAMS-

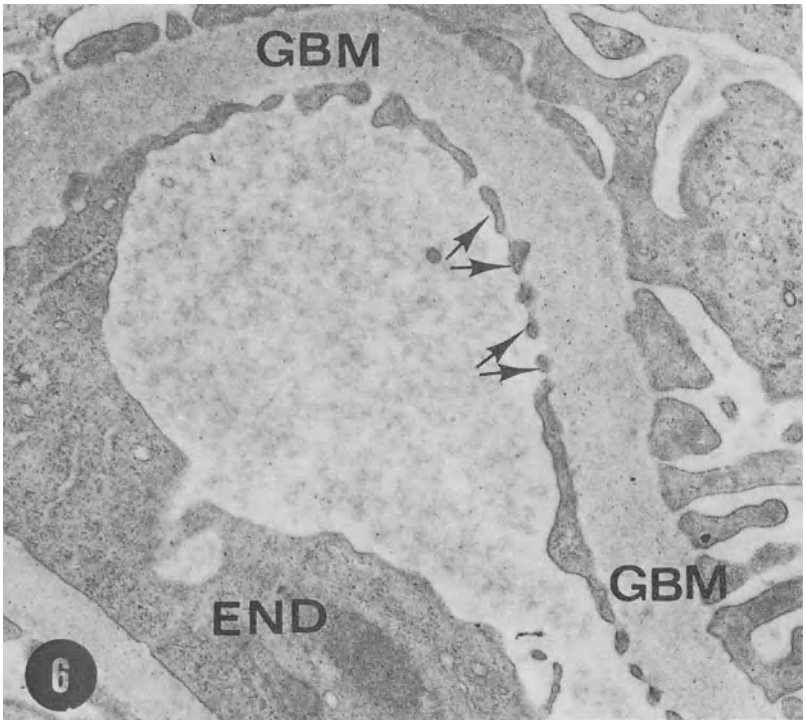

Fig. 5-6. An endothelial cell (END) is attached to a part of the glomerular basement membrane (GBM). Normally the vast majority of the portions of GBM have interrupted attachments of fragments of endothelial cytoplasm called fenestrae (arrows). Between the fenestrae there are gaps that presumably permit the passage of glomerular contents into the urinary space. The endothelial cell has fewer organelles, e.g., endoplasmic reticulum, mitochrondria (UA + LC, ×20,000).

stained section (Fig. 5-5). Most of the foot processes contain no material other than cytoplasm; occasional foot processes contain vacuoles or multivesicular bodies (Fig. 5-4).

Mesangium (Figs. 5-2, 5-3, 5-7 to 5-9)

Mesangium refers to the thin membrane that helps to support the capillary loops in a renal glomerulus. The term mesangium was coined by Zimmermann

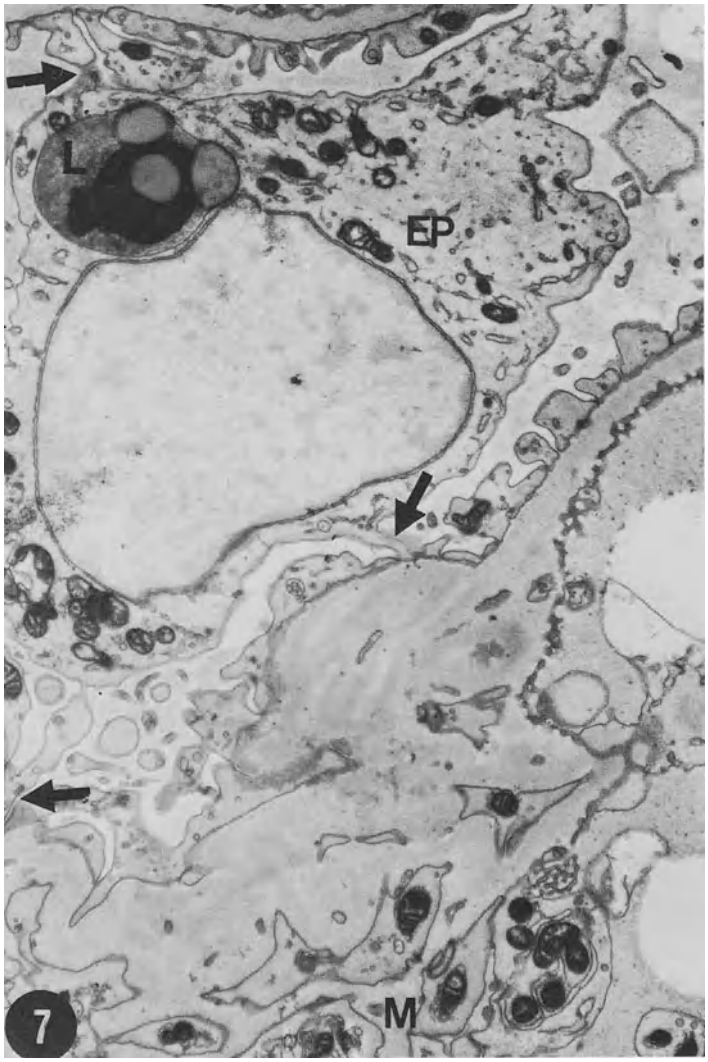

Fig. 5-7. Presence of abundant cell organelles consisting of many mitochondria in the epithelial cell (EP). Note that the EP is attached to three different glomerular capillaries by foot processes (arrow). A lysosome (L) is seen in the epithelial cell. The mesangium (M) is shown (PAMS, ×16,000).

in 1933 (for details see Heptinstall, 1974), who described it as a group of fibroblasts lying between sheets of basement membrane of the capillary loops of glomerulus in a topographic relation similar to the mesenterium of the bowel. The EM study has clarified the anatomy of the mesangium and confirmed the imaginative idea of Zimmermann. Mesangium can now be defined as a space formed by the centrilobular portions of the capillary loops; a cross section reveals a space surrounded by basement membrane-like (BM) materials and containing cells which appear similar to endothelial cells (Figs. 5-3, 5-8, and 5-9). In general, the mesangial cell is separated from the capillary lumen by the intervening endothelial cell and these two cells are almost always bridged by BM materials (Figs. 5-3 and 5-9). In some instances, a continuity of the mesangial cell with the capillary lumen has been observed (Fig. 5-9). The mesangial cell is of various sizes and shapes, and has a nucleus disproportionately large for the cell, a small amount of cytoplasmic material, and a very few cell organelles (Figs. 5-9 and 5-10). A normal mesangium has one to two cells (Figs. 5-3 and 5-10) but it may demonstrate no cells (Figs. 5-8 and 5-10). Individual cells may be separated by BM materials when more than one cell is present. The cells extend cytoplasmic

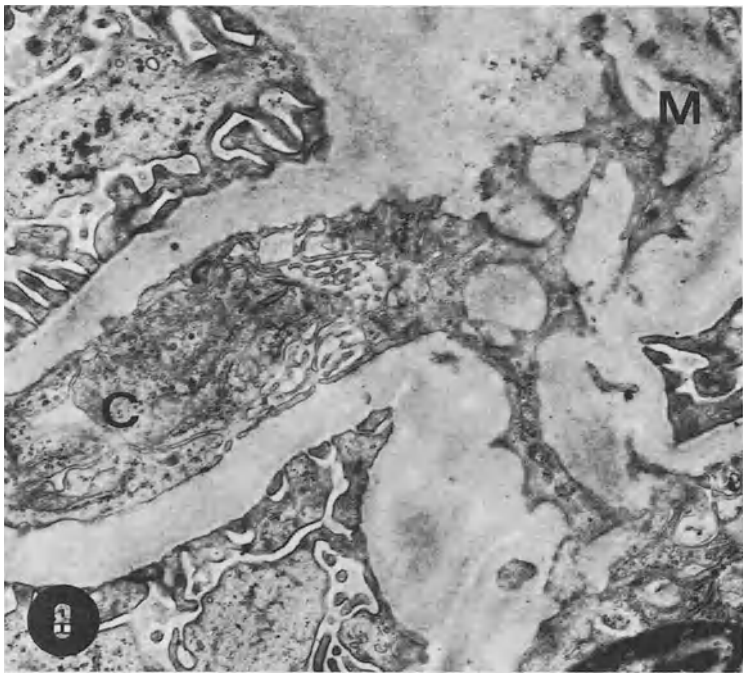

Fig. 5-8. A mesangium (M) bounded by the centrilobular portions of the capillaries. The mesangium has more than anticipated amounts of matrix. The peripheral portion of the capillary loop (C) appears essentially normal. The apparent occlusion of the capillary loop (C) is an artifact. This type of collapse can occur as a result of sudden exposure of the tissue to high beam (UA + LC, ×18,000). This section is from a kidney originally harvested for the purpose of transplantation (21-year-old white male victim of motorcycle accident).

processes toward the centrilobular portions of the capillaries and toward the endothelial cells (Fig. 5-9).

An anatomical connection between mesangium and juxtaglomerular apparatus has been described by an occasional investigator, although I have never observed this connection in my own studies of kidneys from humans, rats, and dogs. The mesangium and the mesangial cell appear to be functional, but the significance of this function (except as a supporting framework for the glomerular capillaries) is unclear. It has been stated that in pathological conditions the mesangial cell proliferates and becomes phagocytic. The phagocytic function of the mesangium and the mesangial cell has been documented by demonstration of colloidal particles in the mesangium a few minutes after an intravenous injection of colloidal particles in rats. Other investigators have shown that injected macromolecules such as heat-aggregated IgG were readily taken up by the mesangium

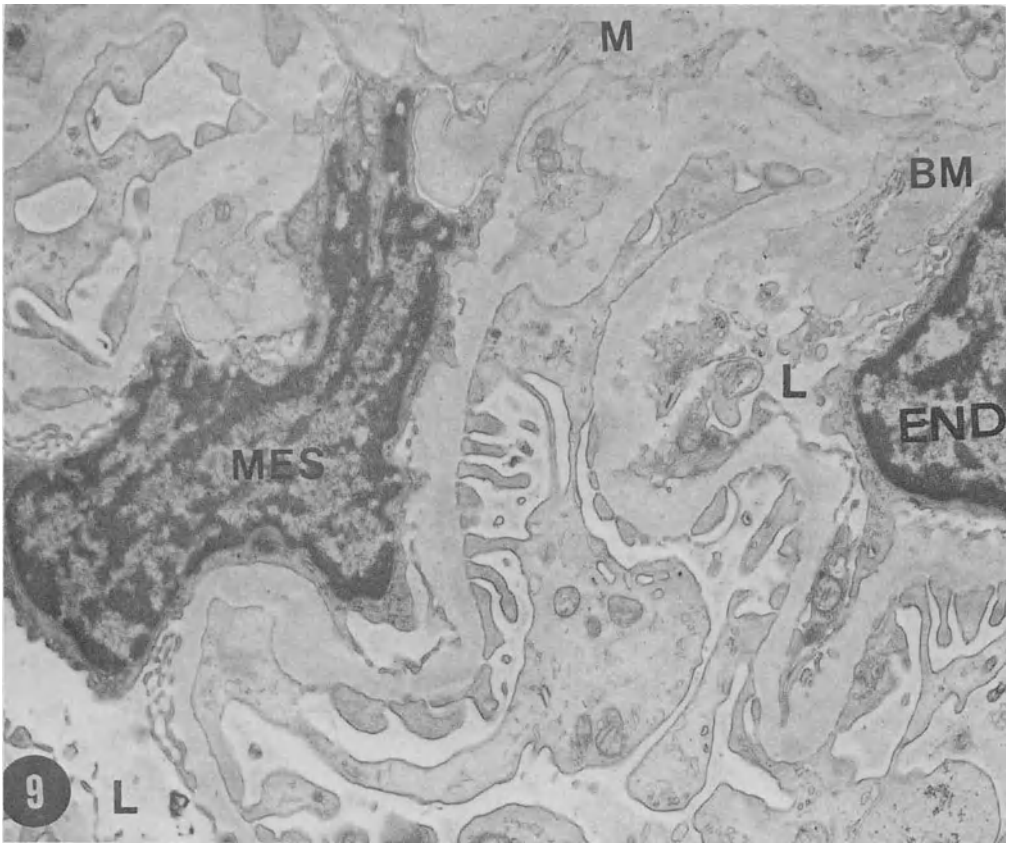

Fig. 5-9. This micrograph demonstrates communications between mesangial cell (MES) and the lumina of the glomerular capillary (L). Also shown is the separation between mesangium (M) and endothelial cell (END) by basement membrane-like material (BM) (UA + LC, ×16,000).

of normal rats and that greatly increased quantities entered the mesangium in experimental nephrotic syndrome and nephrotoxic serum nephritis. The findings of electron-dense deposits in the mesangium in immune complex glomerulonephritis, mesangiopathic GN, IgA GN, and nonstriated fibrils in amyloidosis support this concept. Among other functions, there is some evidence to suggest that the mesangial cell is involved in the production of BM materials.

Bowman's Capsule, Bowman's Space, and Bowman's Epithelium

By light microscopy, Bowman's capsule appears as a thin membrane lined by a single layer of flattened epithelial cells. Bowman's membrane, like tubular

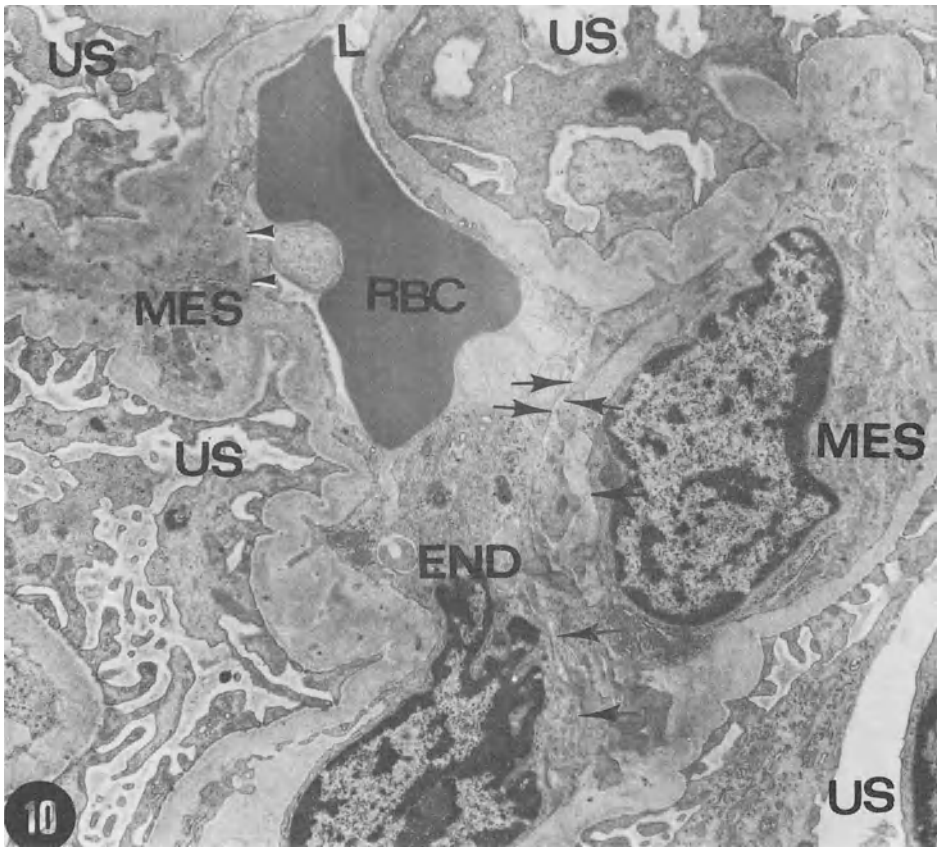

Fig. 5-10. A typical normal mesangial cell (MES) is separated from the endothelial cell (END) by basement membrane-like material (arrows). Mesangium is separated from the capillary lumina (L) by basement membrane-like material (arrowheads). A red blood cell (RBC) is within the lumen. The urinary space (US) is shown (UA + LC, ×14,000).

basement membrane, is observed best in PAS- or PAMS-stained sections (Fig. 5-11). By EM, Bowman's capsule reveals a basement membrane twice as thick as normal GBM, and almost as thick as the basement membrane of proximal tubules. The epithelium has a single layer of epithelial cells which resemble visceral epithelial cells. Between Bowman's (parietal) epithelial cells and visceral epithelial cells a clear space called Bowman's space is always discernible in normal kidneys (Fig. 5-12). This space becomes obliterated by proliferated epithelial cells in pathological states. In a normal condition, however, fragments of epithelial cells, a few mitochondria, and a few microvilli may be found in Bowman's space.

Bowman's membrane is separated from the proximal or distal convoluted tubule by a 0.5- to 1-μm-wide space. This space normally contains no more than a few collagen fibers (see Fig. 11-8b).

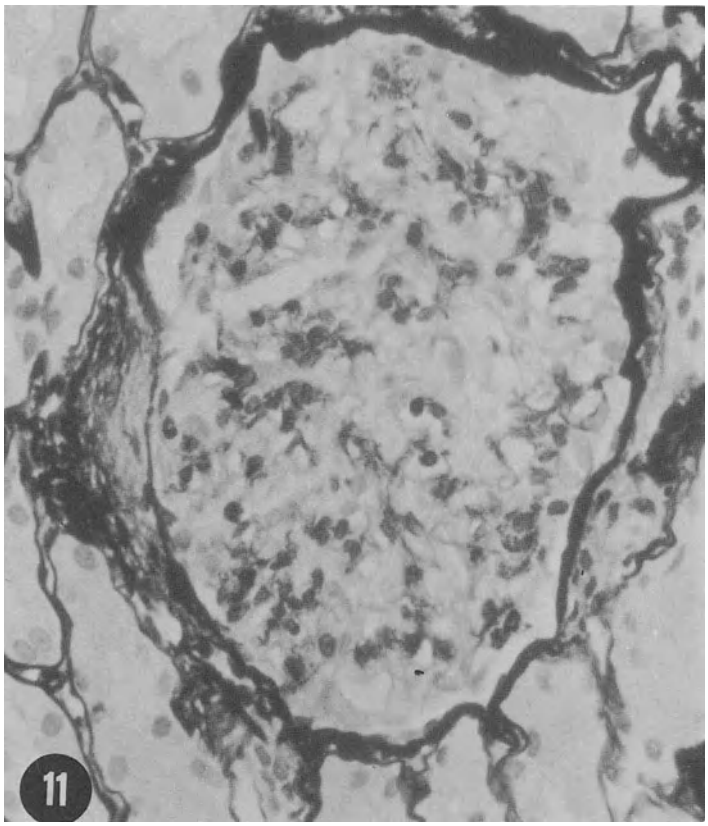

Fig. 5-11. Clear delineation of Bowman's membrane and tubular basement membrane by silver technique and with the use of light microscopy. Normally cortical tubules are apposed to each other and to Bowman's membrane with slight or no discernible space (PAMS, ×320).

The juxtaglomerular apparatus (JGA) is a composite structure; it is roughly triangular in shape and located between the Bowman's membrane, efferent or afferent arteriole and distal convoluted tubule. The cells in the JGA are mainly composed of arteriolar smooth muscle cells (JG cells), which, as has been stressed by numerous individuals, contain granules. In my EM studies of glomeruli in several hundred human normotensive and hypertensive renal biopsies and an innumerable number of kidneys from normotensive and hypertensive rats and

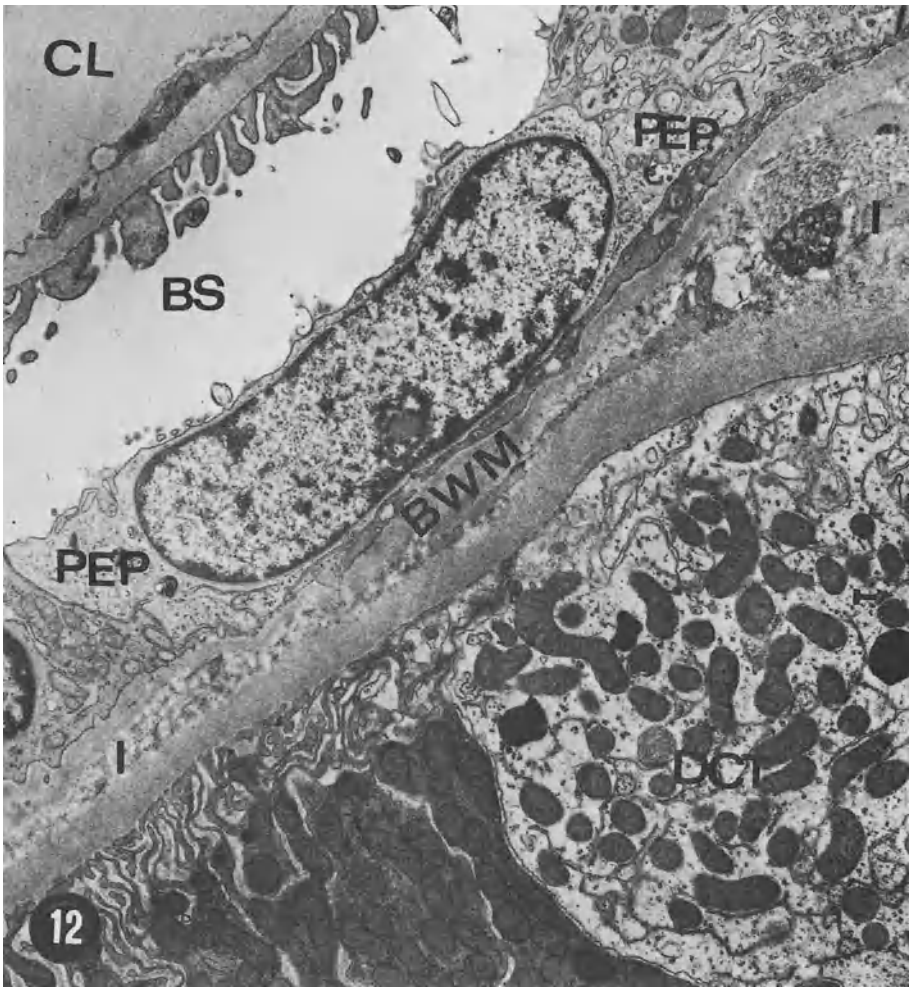

Fig. 5-12. A glomerulus adjacent to a distal convoluted tubule (DCT). Note the interstitium (I), Bowman's membrane (BWM), Bowman's or parietal epithelial cell (PEP), Bowman's space (BS), and the lumen of a glomerular capillary (CL) (UA + LC, ×13,000).

dogs, I have found JGA cells in only a few instances (Fig. 5-13a), and only seldom were they found to contain granules. Thus, at the least, JG cells do not contain granules which are as conspicuous as mast cell granule or papillary interstitial granule. However, JGA in rat kidney is discernible more frequently and contains more granules than JGA in human kidney (Fig. 13-b).

TUBULES

Tubules form the largest bulk of the renal parenchyma. Normally in LM sections, large numbers of tubules are seen apposed to each other and in close apposition with a glomerulus (Fig. 5-11). Many have small lumina, striations at

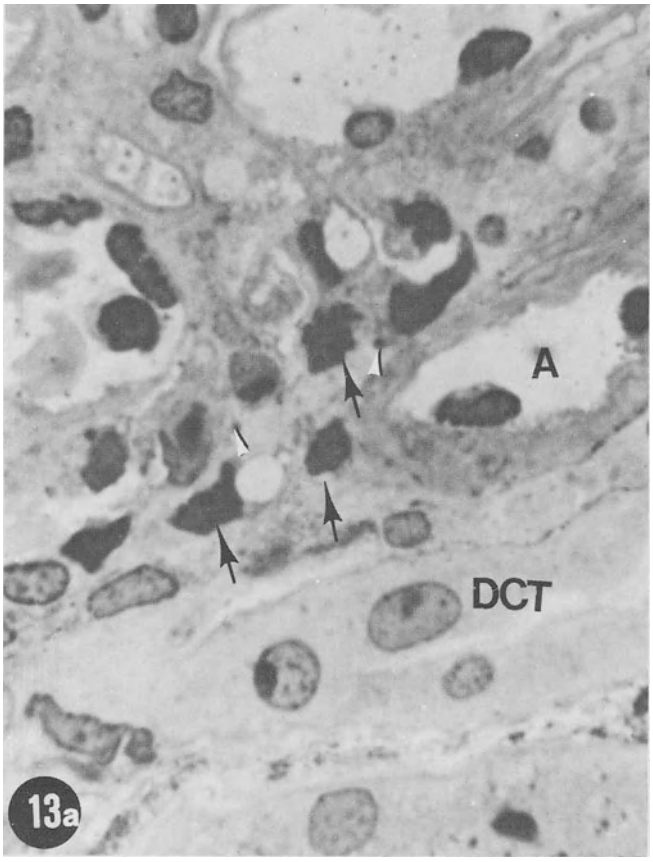

Fig. 5-13a. A juxtaglomerular apparatus is bounded by the glomerular capillaries above, and an arteriole (A) and a distal convoluted tubule (DCT) below. The JGA has demonstrable cells (arrows) but granules (arrowheads) can be discerned only occasionally (methylene blue-azure II, ×800).

the luminal edge, and nuclei located at the middle or base of the cells. The cells rest on a basement membrane which is delineated precisely in PAS- and PAMS-stained sections (Fig. 5-11). Even with careful LM examination it is sometimes difficult to distinguish proximal convoluted tubule from distal convoluted tubule. This is particularly difficult in pathological states.

Since many tubules lie in apposition with a glomerulus, thin sections (sections for EM study) of the glomerulus contain all types of tubules in sufficient number, thus permitting a thorough evaluation of tubules along with glomeruli by EM. EM can distinguish the different types of tubules, i.e. proximal convoluted tubule, distal convoluted tubule, loop of Henle, and collecting tubule. For a comprehen-

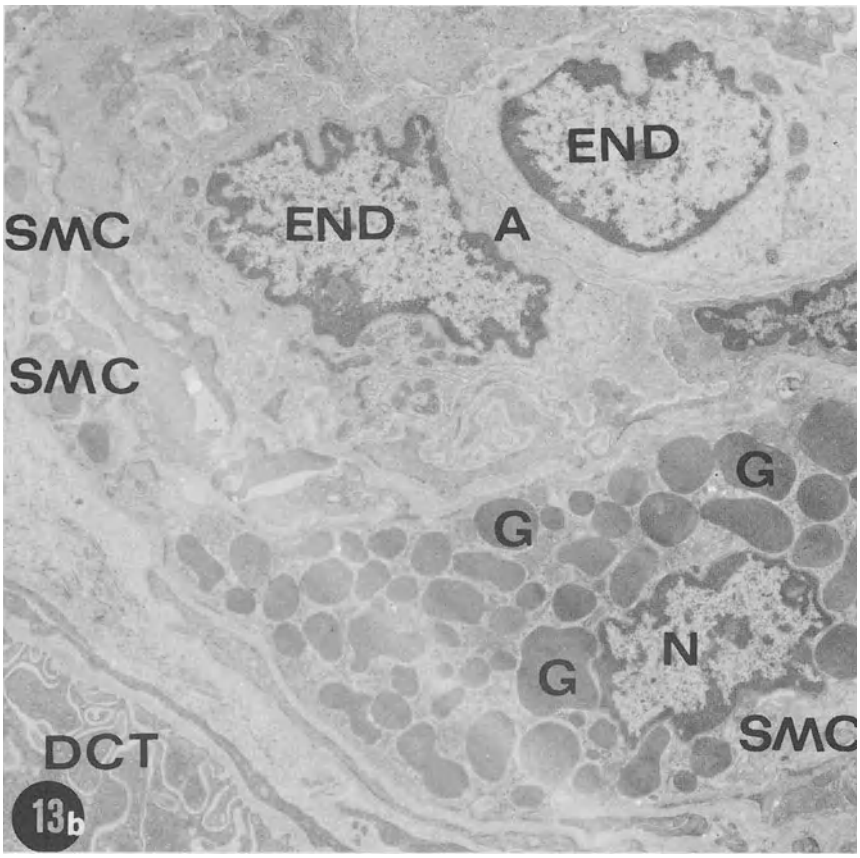

Fig. 5-13b. Electron microscopy of a part of the juxtaglomerular apparatus from a normotensive Wistar rat demonstrates an efferent arteriole (A) and partly a distal convoluted tubule (DCT). The efferent arteriole is recognized by a single but incomplete layer of smooth muscle cells (SMC) and prominent endothelial cells (END) which resemble those in a capillary. The characteristic feature of this SMC is the abundant electron-dense granules (G). The nucleus (N) is small. This unique feature identifies this type of arteriolar SMC as juxtaglomerular cells (renin-secreting cells) (UA + LC, ×7000).

sive study of tubules, thin sections should be collected on gold grids and stained with 1% periodic acid and diluted methylenamine silver (for details see Chapter 2).

The following components are common to all types of tubules: basement membrane, plasma membrane infoldings, nuclei, and mitochondria. The basement membrane (TBM) of proximal convoluted tubule (PCT) is thicker than that of distal convoluted tubule (DCT), Henle's loop (HL), and collecting tubule (CT). Proximal to the basement membrane, numerous infoldings of the basal plasma membrane are seen; these plasma membrane infoldings or folds interdigitate tubular cells and constitute a more conspicuous feature in proximal and distal convoluted tubules than other types of tubules. In these tubules the membrane folds encircle individual mitochondria or group of mitochondria, giving the ap-

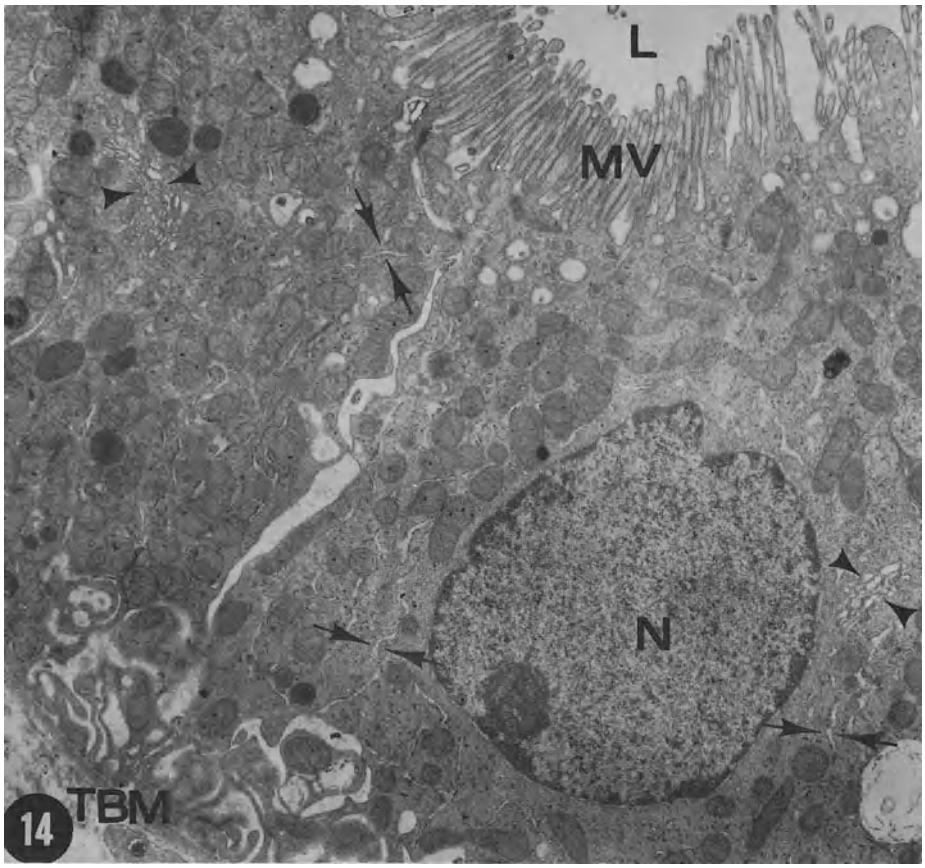

Fig. 5-14. A proximal convoluted tubule. A cross section through the tubule reveals several cells. Each cell has a number of cords which are connected to each other by the plasma membrane folds (arrows). The nucleus (N) is located more toward the base than the apex; the mitochondria are evenly distributed throughout the cells. There are abundant microvilli (MV) at the apical surface. Note prominent Golgi complexes (arrowheads), lumen (L), and basement membrane (TBM) (UA + LC, ×18,000).

pearance of whorls. The anatomy of the tubules is better studied in PAMS- than UA + LC-stained sections.

Proximal Convoluted Tubule (Figs. 5-14 to 5-17)

The proximal convoluted tubule is characterized by bundles of microvilli, broad basement membrane, and numerous mitochondria. The microvilli are located at the apical surface of the cells and appear as straight cylinders called brush border. Close observation reveals thin membranes surrounding these cylinders (Figs. 5-14 and 5-15). These membranes are apposed to each other, giving a double membrane appearance between the cylinders (Figs. 5-16 and 5-17). Small vesicles which are pinched off apical pits may be seen at the base of the microvilli (Figs. 5-16 and 5-17). The apical vesicles may fuse to produce vacuoles (Figs. 5-16 and 5-17). Many lysosomes, which may be the product of apical

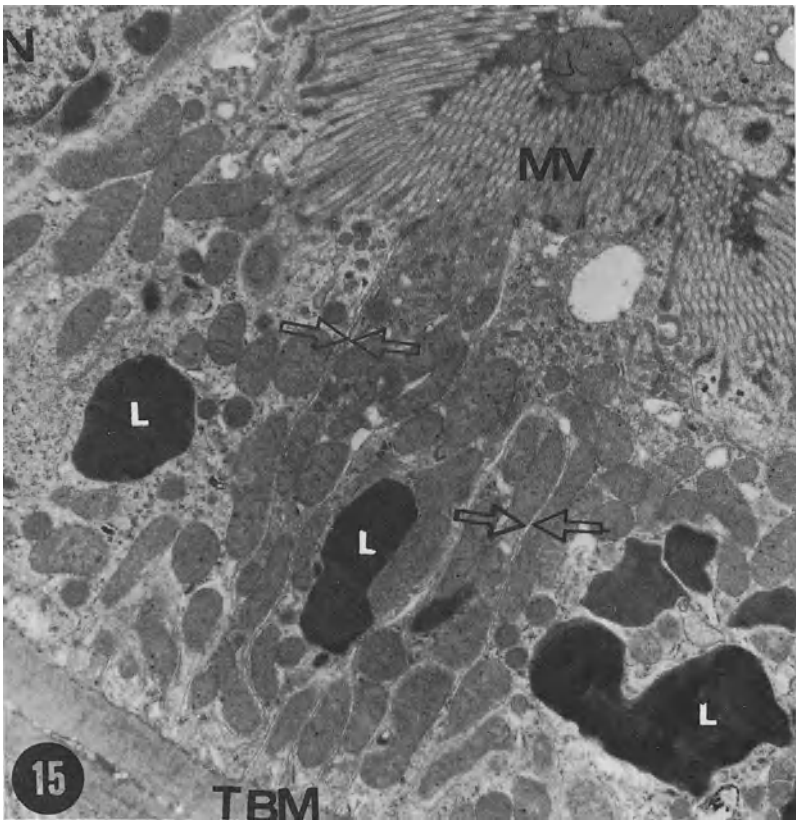

Fig. 5-15. A proximal convoluted tubule from a normal dog reveals features similar to those in the proximal tubule from a man (Fig. 5-14) except there are more lysosomes (L) in dog than in the human (UA + LC, ×20,000).

vesicles and vacuoles, are found in proximal convoluted tubule, especially in dog (Fig. 5-15). The nucleus is spherical and commonly located more toward the base than the apex (Figs. 5-14 and 5-16), although this location varies, and is therefore not a feature that should be used to distinguish between the tubules. The mitochondria are numerous, consistent in size and shape, and found in all regions of the cell except just beneath the apical surface. They are mostly oriented in the long axis of the cell, and assessed ideally in PAMS-stained sections (Figs. 5-16

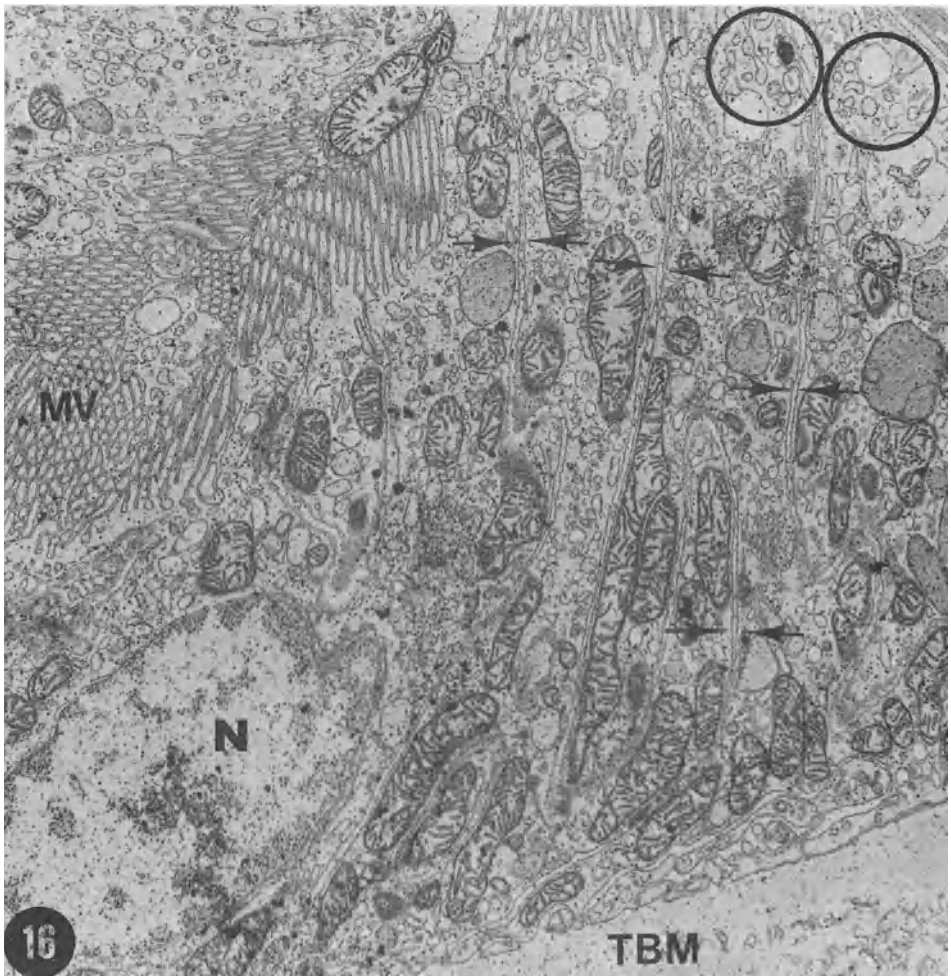

Fig. 5-16. A proximal convoluted tubule from a dog studied by silver technique clearly delineates the plasma membrane infoldings (opposing arrows), mitochondria along the long axis of the cell, and apposition of microvilli (MV), giving a double membrane appearance. Note the collection of small vesicles which represent the apical pits of microvilli (circles). Tubular basement membrane (TBM) and nucleus (N) of the tubule are shown (PAMS, ×19,000).

to 5-17). It is understood that the presence of numerous mitochondria is necessary to provide a source of energy to facilitate the active transport function of the proximal convoluted tubule.

Distal Convoluted Tubule (Figs. 5-18a and 5-18b)

Distal convoluted tubule is differentiated from proximal convoluted tubule essentially by (1) the presence of a few or no microvilli, (2) location of the nucleus toward the apical surface of the cell, (3) a relatively small number of mitochondria

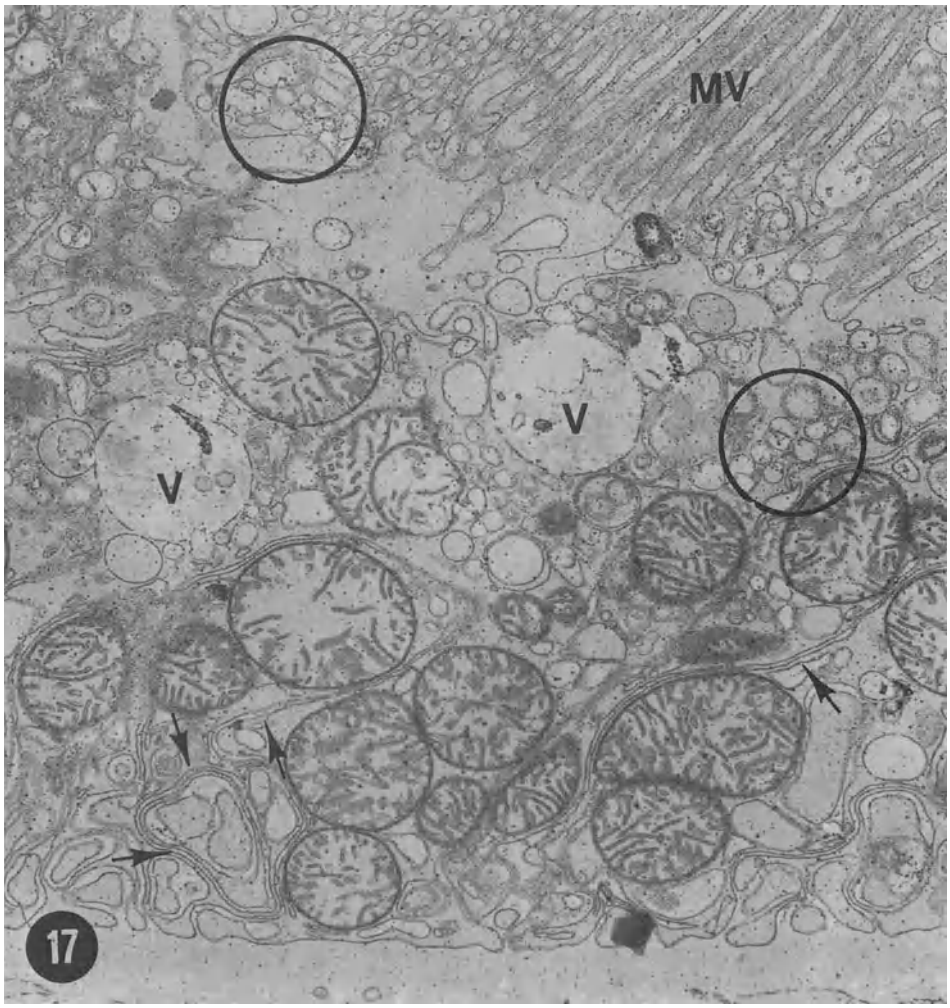

Fig. 5-17. A magnified view of dog's proximal tubule clearly reveals basal infoldings (arrows), microvilli (MV) with double membrane appearance, apical vesicles (circles), and vacuoles (V) (PAMS, ×23,000).

located mainly toward the basal surface of the cell, and (4) more prominent basal infoldings.

Loop of Henle (Fig. 5-19)

The cells of the loop of Henle are usually conical with narrow apices and broad bases. The cell has a relatively large nucleus and a few cell organelles. There are few microvilli at the apices of the cells.

Collecting Tubule (Fig. 5-20)

The collecting tubule consists of dark and pale cells, the dark cells containing more mitochondria and endoplasmic reticulum than the pale cells. The cells are generally elongated and demonstrate a few rudimentary microvilli at the apical

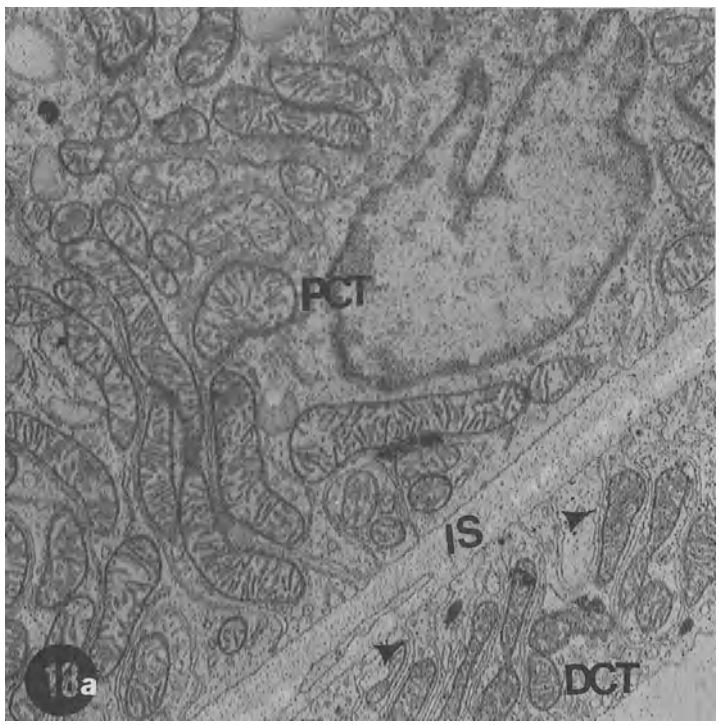

Fig. 5-18. (a) Dog kidney, demonstrating adjacent proximal convoluted tubule (PCT) and distal convoluted tubule (DCT). Note more prominent basal infoldings (arrowheads) in DCT than PCT and absence of microvilli in DCT. Inconspicuous interstitial space (IS) is the usual finding (PAMS, ×18,000). (b) Magnified view of distal convoluted tubule (DCT) from a human subject. Note basement membrane of the tubule (TBM), location of the nucleus (N) toward the apex, and conspicuous folds of plasma membrane surrounding groups of mitochondria (PAMS, ×20,000).

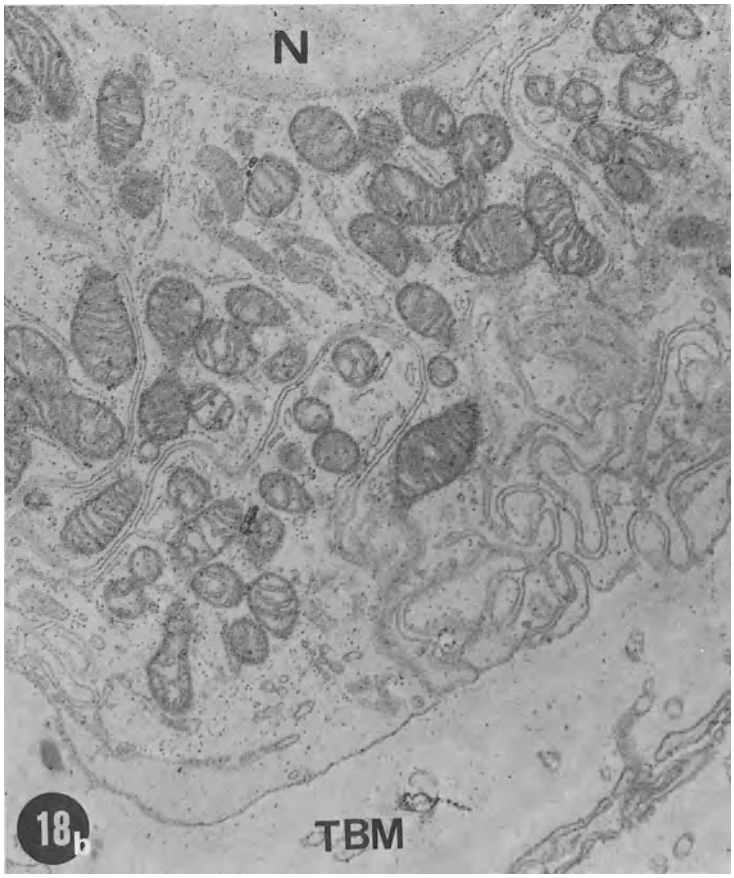

Fig. 5-18. *(Continued)*

portions. The plasma membrane infoldings are located mainly at the basal portions of the cells and do not interdigitate the individual epithelial cells as much as in the proximal and distal tubular epithelial cells. The morphology of the collecting tubule varies somewhat according to the location, i.e., corticomedullary junction, medulla, or papilla. The collecting tubules are most easily distinguishable in the papilla due to the intervening wide interstitium and interstitial cells separating them from loop of Henle or peritubular capillaries.

ARTERIAL VESSELS (Figs. 5-21 to 5-27)

Three types of arterial vessels can be recognized by EM study: small artery, large arteriole, and small (terminal) arteriole, and each vessel type exhibits three definite layers: endothelial, basement membrane, and medial (smooth muscle

cell). The layers of endothelial cells and smooth muscle cells are of little value in differentiating between the different types of vessels. Smooth muscle cells (SMC) appear to differ, however, in that those of the small artery are relatively large with comparatively few organelles (Fig. 5-21), whereas those of the large arteriole are generally smaller and apparently more active, with many mitochondria and other organelles (Fig. 5-22). The smooth muscle cells in the small (terminal) arteriole are single-layered and have fewer organelles (Figs. 5-23 and 5-24).

In the histologic sections studied by LM it is frequently difficult to distinguish between the three types of vessels. A small artery can be distinguished from a small arteriole via EM study, by the presence of a broad lamina between en-

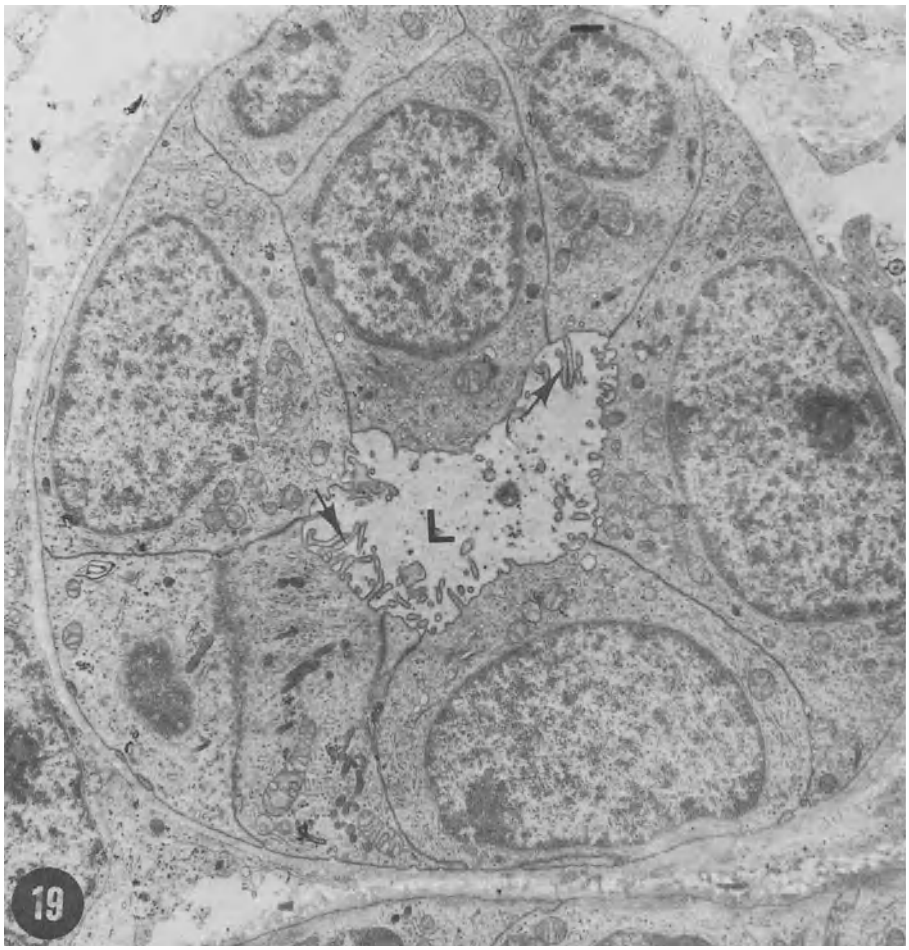

Fig. 5-19. Loop of Henle. Note the few microvilli (arrows) and lumen (L) of the tubule (UA + LC, ×5000).

dothelial cell and smooth muscle cell and more than one layer of SMC (Fig. 5-21 versus Fig. 5-24). Since the small artery and the large arteriole may appear alike, however, it is frequently difficult to separate the two, a problem that has confronted many investigators. Movat and Fernando (1963) have emphasized that the presence or absence of internal elastic lamina is an important criterion for distinguishing arteries from arterioles. Small arteries are reported to have an internal elastic lamina, whereas the small or terminal arterioles do not. We have demonstrated by using silver tetraphenyl porphyrin sulfonate (STPPS), a specific electron-dense stain for elastic tissue, that arterial vessels of all sizes contain variable amounts of elastic tissue. (For details of STPPS stain, see Chapter 2.) On the basis of our findings, we have suggested that the thickness of the basement membrane intervening between endothelial cells and smooth muscle cells with variable contents of elastic tissue appears to be the most valuable index for differentiating arterial vessels of different sizes. The proportion of elastic tissue in the basement membrane decreases in the following order: small artery, large arteriole, and small arteriole. Thus, a small artery has a thin basement membrane

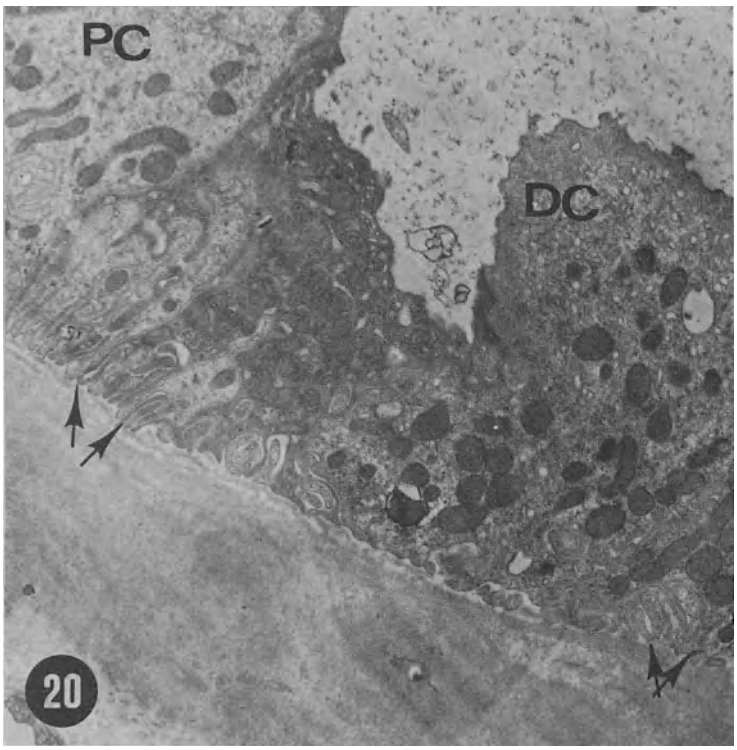

Fig. 5-20. Collecting tubule. Note pale cells (PC) and dark cells (DC). Basal folds are quite prominent (arrows) (UA + LC, ×17,000).

containing bulk elastic tissue (Fig. 5-25); in contrast, a small arteriole has a more conspicuous basement membrane containing much less elastic tissue (Fig. 5-26). In the large arteriole the basement membrane is intermediate in thickness and elastic tissue content (Fig. 5-27). The peritubular capillary shows a few specks of elastic tissue.

Although the arterial vessels in normal animals have been studied by EM extensively, information on the fine structure of renal arterial vessel in normal humans is still lacking. So far, ultrastructural observation of the human renal arterial vessels has been made in hypertension and renal disease. Considering the little information available, renal arterial vessels in normal subjects resemble those in normal rats and dogs; however, some differences exist: (1) Elastic tissue content is comparatively smaller in amount than that in rats or dogs; and (2)

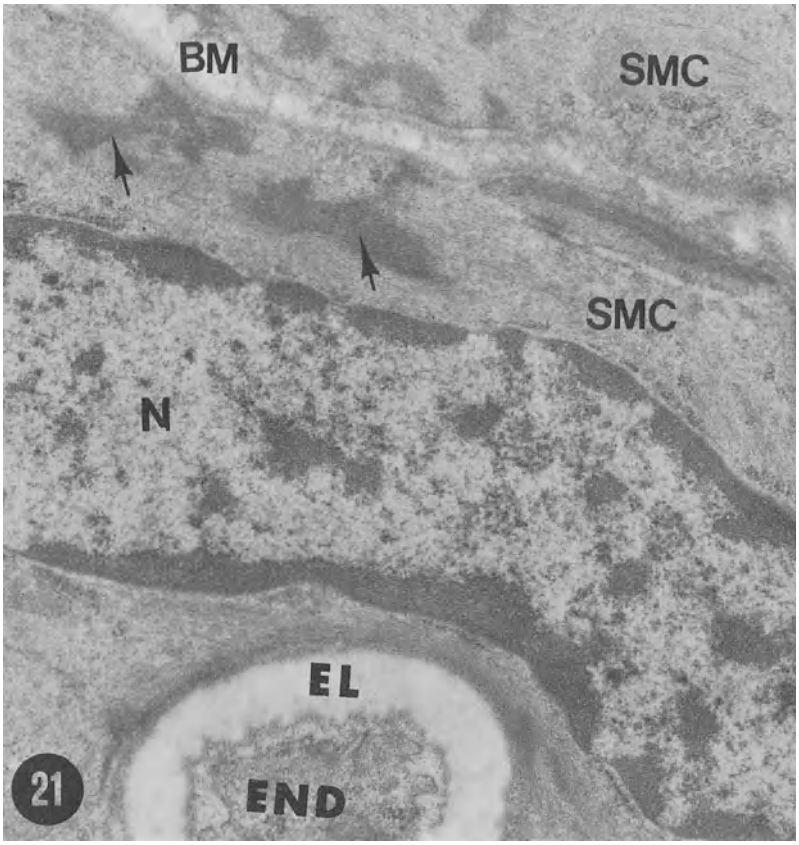

Fig. 5-21. A small artery from a dog kidney demonstrates thick band of electron-lucent material (EL) consistent with elastic tissue between endothelial cell (END) and smooth muscle cell (SMC). This EL has been described as internal elastic lamina by numerous individuals. Layers of smooth muscle cells (SMC), nucleus (N) of smooth muscle cell, attachment plates (arrows) of smooth muscle cells, and the basement membrane (BM) between individual smooth muscle cells can be seen (UA + LC, ×23,000).

basement membranes between endothelial cells and smooth muscle cells and between individual smooth muscle cells appear to be comparatively thicker than those in rats and dogs.

INTERSTITIUM (Figs. 5-18a, 5-28 to 5-30)

The interstitium is a space bounded by Bowman's membrane and cortical tubules; cortical tubules (cortical interstitium); medullary tubules (medullary interstitium); and collecting tubule, Henle's loop, and peritubular capillaries (pap-

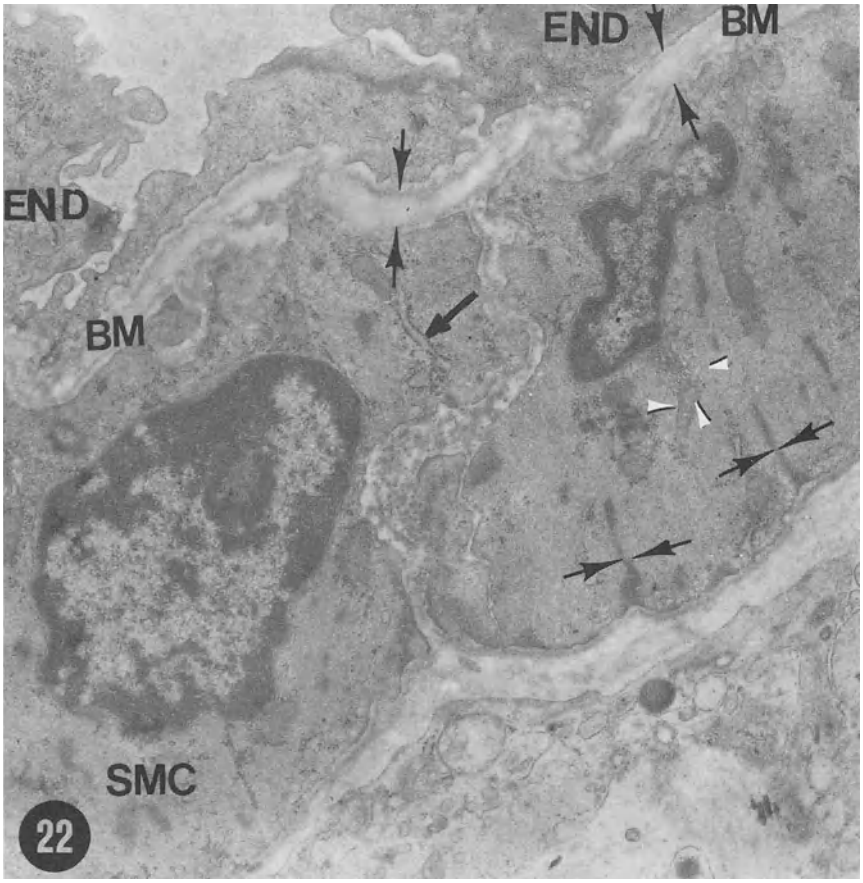

Fig. 5-22. A large arteriole from a normotensive rat. Note in the smooth muscle cell (SMC) rough-surfaced endoplasmic reticulum (arrow), Golgi complex (arrowheads), many mitochondria, and attachment plates (opposing arrows). Within the basement membrane (BM) between endothelial cell (END) and SMC, segments of electron-lucent material represent elastic tissue (opposing arrows) (UA + LC, ×21,000).

illary interstitium). The interstitium is thin and inconspicuous in the cortex (Figs. 5-18b and 5-28), prominent in the papilla (see Figs. 9-43 and 9-44) and intermediate in the medulla. The human or dog kidney has six papillae, whereas the rat kidney has a single papilla, and consequently the papillary interstitium is widest and most conspicuous in rats (see Figs. 9-43 and 9-44). Normally the cortical interstitium contains no more than a few collagen fibers and occasional fibroblasts (Figs. 5-18b and 5-28) and mast cells (see later). Under normal conditions the medullary and papillary interstitium contains moderate amounts of collagen fibers, a few fibroblasts, and conspicuous interstitial cells (see Figs. 9-43 and 9-44). The anatomy of the interstitial cell is described in detail in Chapter 9 because of the implication of interstitial cell in hypertension.

The other characteristic cell in the interstitium is the mast cell. It is very difficult to recognize this cell by convential LM study; therefore, it is studied

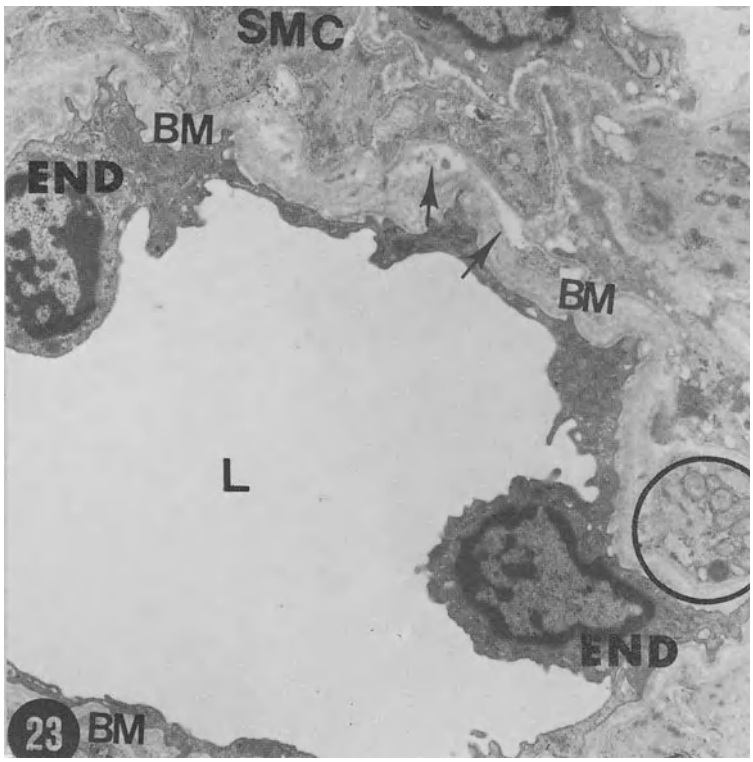

Fig. 5-23. A small arteriole from human kidney demonstrates prominent basement membrane (BM) between endothelial cell (END) and smooth muscle cell (SMC); small amounts of electron-lucent materials are presumably elastic tissue (arrows). Note many mitochondria (circle) in the SMC. Lumen (L) of the arteriole is shown (UA + LC, ×13,000).

most conveniently by EM. There are two types of mast cells: ovoid and spindle (Figs. 5-29 and 5-30). Either type is characterized by villous projections on the surface of the cell, a single nucleus of variable size and shape, rare mitochondria, sparse endoplasmic reticulum, and the most conspicuous feature, abundant granules. The normal kidney exhibits few, if any, mast cells, whereas the pathological kidney contains a large number. The granules in the mast cells are considered to be the most important histological feature and presumably the very basis of mast cell biological activity. There are two types of granules, smooth homogeneous granules called immature granules (Fig. 5-29) and coarse granules associated with whorls or cylinders called mature granules (Fig. 5-30). Each cell type has uniform granularity, i.e., immature or mature. Free granules are observed outside the cell and appear to be actively discharged by the cell (Fig. 5-30). An increased number of mast cells have been observed in pathological kidneys, especially chronic pyelonephritis and malignant hypertension. Since the mast cell is known to

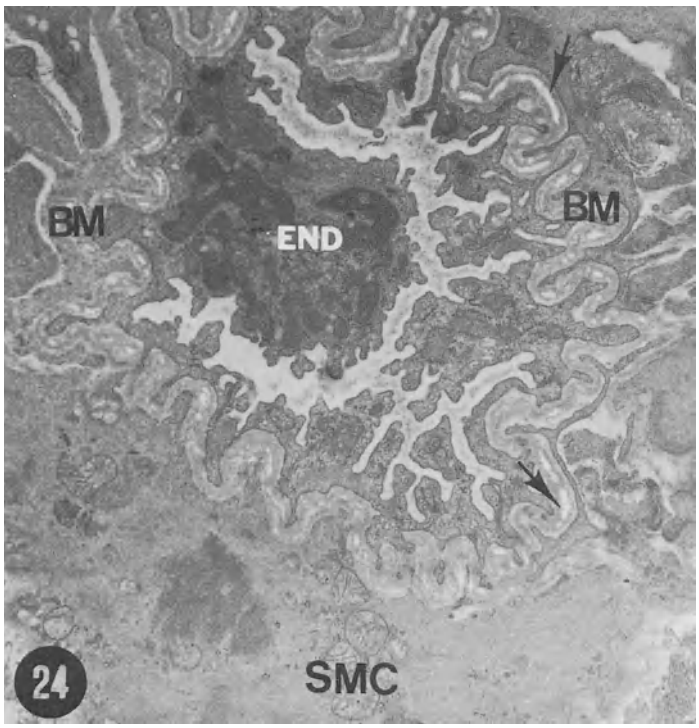

Fig. 5-24. A small arteriole from a normotensive rat demonstrates curled-up basement membrane (BM) with a small amount of electron-lucent material that is presumably elastic tissue (arrows) and a single layer of smooth muscle cells (SMC). Note many mitochondria in the SMC. Endothelial cell (END) is shown (UA + LC, ×10,000).

secrete heparin and vasoactive amines, it constitutes an important area for investigation in order to explore the cause and effect relationship between the pathology of the kidney and the presence of an excessive number of mast cells.

From the clinical standpoint, interstitium was the forgotten component of the kidney until a decade ago when hypersensitivity acute interstitial nephritis induced by methicillin and other drugs was recognized. We now believe that renal interstitium is a target area and may become involved in allergic, hypersensitive, infectious, or other processes.

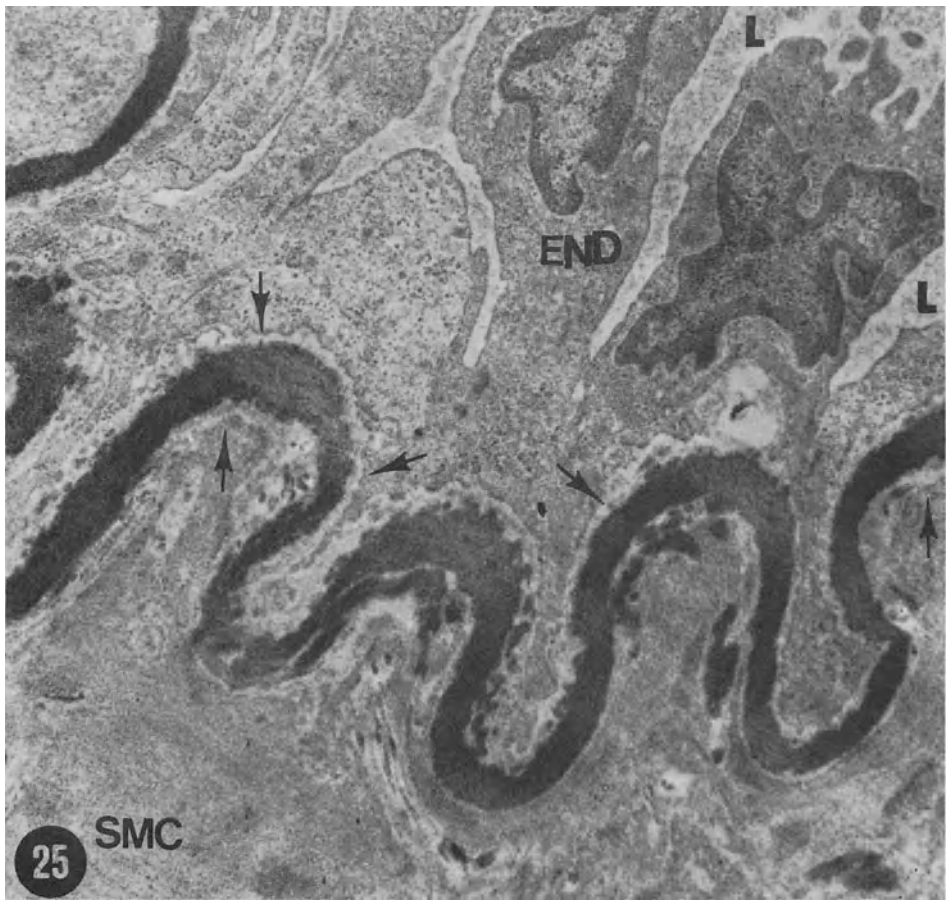

Fig. 5-25. In this small artery from a dog kidney a wide band of electron-dense elastic tissue is seen. On the inner and outer aspects note thin layer of basement membrane (arrows) which has remained unstained by this technique. Other components, i.e., endothelial cell (END) or smooth muscle cell (SMC), exhibit low contrast (STPPS, ×23,000). The band of electron-dense elastic tissue corresponds to the electron-lucent band shown in Fig. 5-21.

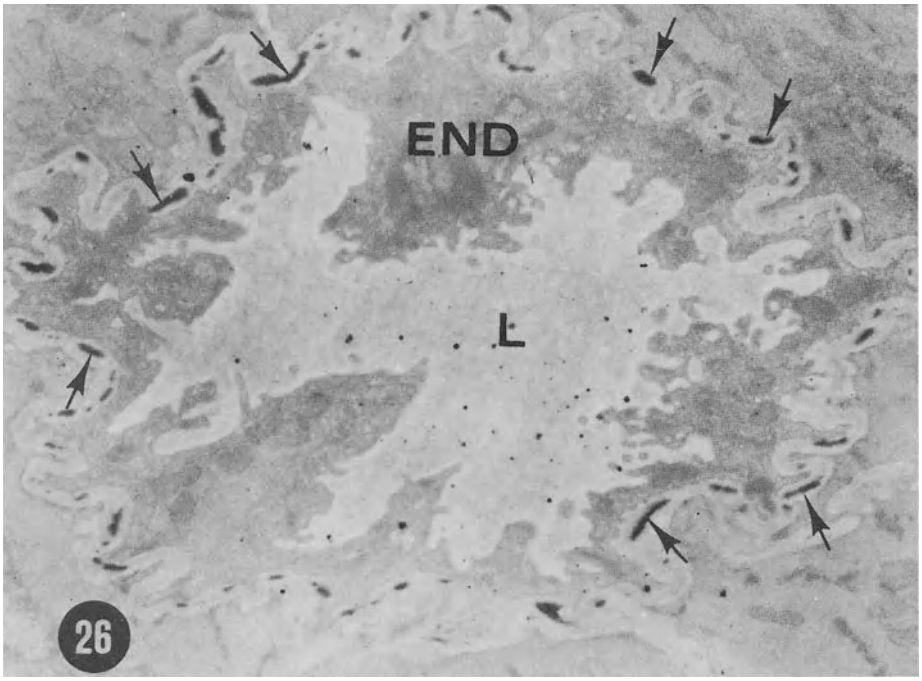

Fig. 5-26. In this small arteriole (similar to that shown in Fig. 5-24), small segments of electron-dense elastic tissue (arrows) are seen corresponding to the electron-lucent materials shown in Fig. 5-24. Lumen (L) of the arteriole and endothelial cell (END) are shown (STPPS, ×8000).

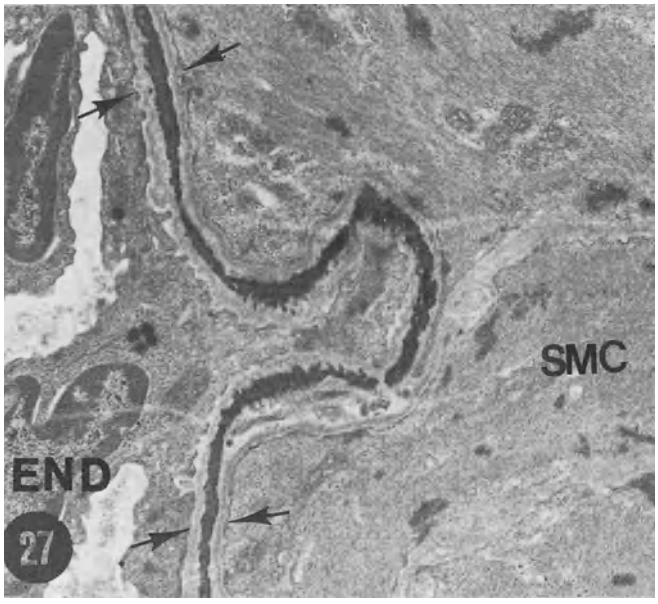

Fig. 5-27. In this large arteriole electron-dense elastic tissue occupies the middle half of the basement membrane between endothelial cells (END) and smooth muscle cells (SMC). Note inner and outer aspects (opposing arrows) of the basement membrane (STPPS, ×12,000).

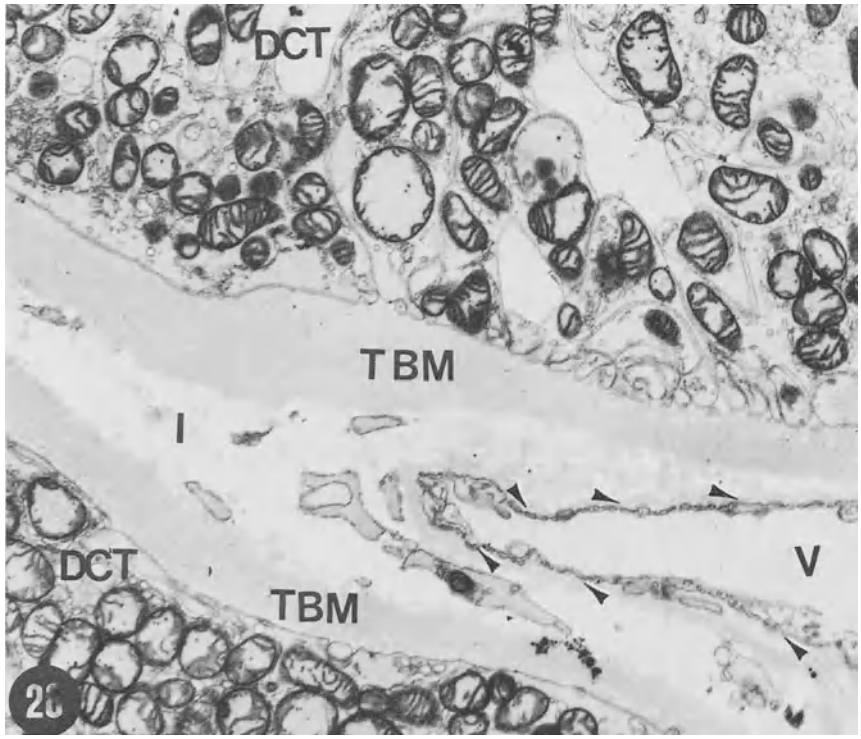

Fig. 5-28. A cortical interstitium (I) is shown between the distal convoluted tubules (DCT). The tubular basement membranes (TBM) are silver positive. A vein (V) (outlined by arrowheads) located between the tubules is also shown (PAMS, ×18,000).

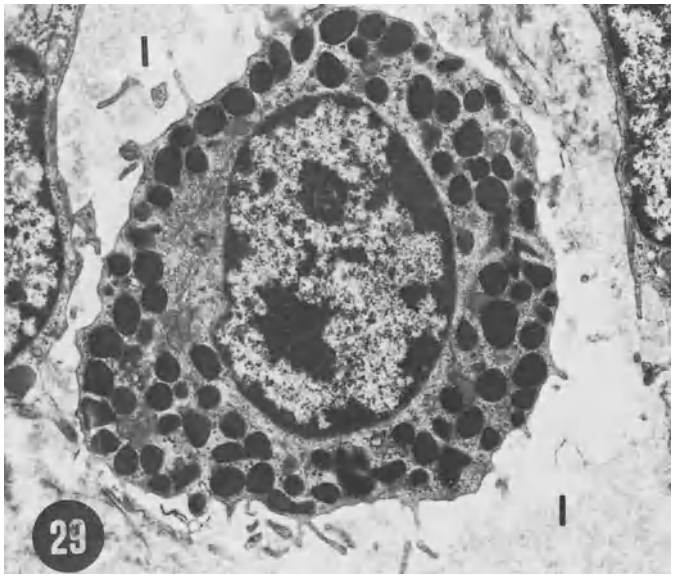

Fig. 5-29. This oval-shaped mast cell located in the interstitium (I) demonstrates intact membrane and one type of granule exclusively. These granules ("immature" granules) are smooth and homogeneously dark (UA + LC, ×11,000).

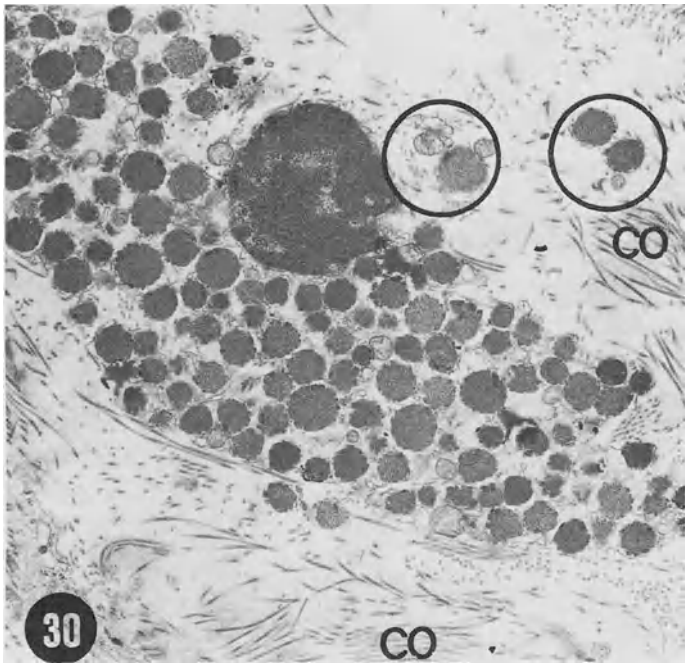

Fig. 5-30. This spindle-shaped mast cell is located in a renal interstitium that contains large amounts of collagen fibers (CO). The mast cell is devoid of membrane and contains coarse granules which are studded with whorls. Because of lack of membrane, many granules are extruded free into the interstitium (circles) (UA + LC, ×15,000).

SUMMARY

1. A comprehensive outline of the electron microscopic study of the different components of the kidney is presented.

2. Broad aspects of the anatomy of the glomerulus, different types of tubules, arterial vessels, and interstitium are described.

3. Finer details of the glomerulus or the various segments of the tubules, e.g., pars convoluta, pars recta, have been omitted so as to provide an overview of the anatomy to medical students and physicians for the purpose of clinical practice.

4. Attempts have been made to clarify long-standing ambiguities in recognizing arterial vessels of different sizes.

5. The detailed morphology of the interstitial cells has been deferred until Chapter 9 because of the implication of these cells in spontaneous or essential hypertension.

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6 Anatomic Pathology of Glomerulonephritis

INTRODUCTION

Glomerulonephritis (GN) is a complex subject to study and understand. The complexity has emerged from a combination of numerous perplexing features of this pathological entity: (1) lack of unanimity in the classifications of glomerulonephritis, (2) our persistent inability in most cases to identify the direct cause or causes of glomerulonephritis, (3) limitations of light microscopy (LM) resolution in delineating finer changes, (4) wide variability in the interpretations of light microscopy findings by different individuals, and (5) the narrow scope of electron and immunofluorescence microscopy techniques around the world.

During the last 10 to 15 years, we have made significant advances in understanding the detailed changes of the kidney in glomerulonephritis via extensive use of electron microscopy in the study of renal tissues. Notwithstanding these improvements in our knowledge, we have made little progress toward establishment of the causative factor or factors which initiate the morphological changes observed in glomerulonephritis. In this respect, our ideas are still nebulous and largely unconfirmed. We have also been unable to demonstrate exact mechanisms of initiation and culmination of glomerulonephritis. This combination of perplexing and unanswered questions has led to many hypotheses about the etiology and pathogenesis of glomerulonephritis. Some of these hypotheses are based on logic, others on fact and still others on neither of these.

The purpose of this chapter is to provide the reader with a comprehensive treatise on the anatomic pathology of glomerulonephritis. The pathological changes are described concisely and the pertinent findings are illustrated in the accompanying figures. At the beginning, it should be stated that the electron microscopy characteristics of glomerulonephritis may at times be confused with

those of glomerulosclerosis. The features distinguishing between glomerulonephritis and glomerulosclerosis are listed in Table 6-1. As already described in Chapter 4, GN can be separated with the aid of electron microscopy study into three definite types and one less precise type: (1) proliferative, (2) membranous, (3) mesangioproliferative, and (4) sclerosing (less precise). The existence of the mixed proliferative and membranous, or membranoproliferative, type is highly controversial. This type is considered by most to be no different from mesangioproliferative glomerulonephritis. There are, however, exceptions, an important one being the membranous transformation of proliferative GN, which has been documented by serial biopsies in lupus GN.

In 80 to 85% of glomerulonephritides, the glomerular fine structures would conform to one of these four types. In 15 to 20% of glomerulonephritides, the ultrastructural findings are subtle, e.g., a slight irregularity of glomerular basement membrane, more on the endothelial than on the epithelial aspect, a mild increase in mesangial matrix, and slight proliferation of epithelial and/or endothelial cells. In this connection, the sampling error of electron microscopy (EM) study must be remembered; these nonspecific changes might be an indicator of

Table 6-1
Differences in the Ultrastructural Characteristics of Glomerulonephritis and Glomerulosclerosis

Criteria	Glomerulonephritis[a]	Glomerulosclerosis
Glomeruli		
Basement membrane		
Integrity	Disruption, thickening, and/or spiking	No disruption, smooth irregular thickening, often tortuous or curled up
Appearance or electron density	Heterogeneous; normal, electron-dense, electron-lucent, or combination thereof	Homogeneous; normal electron density
Electron-dense deposits	Most common finding	Conspicuously absent
Epithelial cells	Variable changes: proliferative, hyperplastic, or atrophic	Often atrophic
Foot processes	Segmental fusion, complete fusion, disappearance	Segmental fusion only
Endothelial cells	No change or proliferative change	Often no change; may be atrophic
Exudation of polymorphonuclear leukocytes	Very common, mild to severe degree	Absent
Mesangium	Excessive cells, excessive matrix, and/or electron-dense deposits	Excessive matrix, atrophic cells, and no deposit
Other components, e.g., tubules, interstitium, and arterial vessels	Sometimes abnormal; the abnormalities include electron-dense deposits in the tubules and arterial vessels and cellular infiltration in the interstitium	Frequently abnormal; atrophic changes in the tubules, cellular infiltration in the interstitium and basement membrane, thickening or deposits in the arterial vessels

[a] Excludes lipoid nephrosis but includes focal glomerular sclerosis.

a focal glomerulonephritis or focal glomerular sclerosis. Since one or two glomeruli are studied, usually by EM, this study alone cannot determine the focal or generalized nature of the disease process. EM, however, can confirm the segmental or diffuse nature of the lesions.

For the purpose of clarity, nomenclature describing the distribution of lesions within individual glomeruli and among all the glomeruli in light microscopy sections are now defined:

1. *Local or segmental*: A portion of the glomerulus and not the whole glomerulus is affected.
2. *Diffuse*: The whole glomerulus is involved.
3. *Focal*: A few of the total number of glomeruli seen in the section reveal the lesion.
4. *Generalized*: All the glomeruli in the section demonstrate lesions.

It should be mentioned that it is common practice to use the term *diffuse* lesions to denote involvement of all glomeruli, sometimes meaning involvement of all components of the kidney, i.e., glomeruli, tubules, arterial vessels, and interstitium.

DIFFUSE (GENERALIZED) PROLIFERATIVE GLOMERULONEPHRITIS

This term means that all the glomeruli reveal hypercellularity with or without exudation. Diffuse proliferative glomerulonephritis can be subdivided into (1) endocapillary proliferative GN; (2) extracapillary proliferative GN; (3) mesangioproliferative GN; and (4) mild proliferation of all cellular elements, i.e., endothelial cell, epithelial cell, and mesangial cell. The separation of diffuse proliferative glomerulonephritis into these types has distinct advantages because clinically they differ in severity, course, and management.

DIFFUSE ENDOCAPILLARY PROLIFERATIVE GLOMERULONEPHRITIS

This is the most common type of glomerulonephritis and generally is associated with immune complex deposition. It is characterized by marked proliferation of endothelial cells, mild to moderate proliferation of mesangial cells, and slight or no proliferation of epithelial cells, and is accompanied almost always by variable exudation of polymorphonuclear leukocytes (PMN) and sometimes segmental necrosis. It may occur as a complication of the following clinical conditions: (1) streptococcal pharyngitis or pyoderma with group A, types 4, 12, 25, and 49; (2) systemic lupus erythematosus (SLE); (3) polyarteritis nodosa; (4) Wegener's granulomatosis; (5) acute or subacute bacterial endocarditis; (6) Henoch–Schonlein syndrome; (7) thrombotic thrombocytopenic purpura (TTP); (8) hemolytic uremic syndrome (HUS); (9) secondary syphilis; (10) sickle cell ane-

mia; (11) malaria; (12) viral infection, e.g., infectious hepatitis, hepatitis associated antigen (HAA), varicella; (13) gammopathy and essential cryoglobulinemia; (14) rheumatic heart disease; (15) chronic lung disease; (16) staphylococcal infection of ventriculoatrial shunt; and (17) visceral abcesses, as well as the use of such drugs as penicillin and penicillamine. (The isolated organisms were identified as *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli,* and *Proteus mirabilis.*)

In polyarteritis nodosa, SLE, Wegener's granulomatosis, bacterial endocarditis, and Henoch–Schonlein syndrome, the renal morphology may reveal more necrosis and exudation than cellular proliferation. This type of renal pathology is frequently described as necrotizing glomerulonephritis. In TTP and HUS, thrombi in the afferent arteriole and glomerular capillaries are the predominant finding. The features of endocapillary proliferative glomerulonephritis as distinguished by light microscopy in the setting of different conditions are listed in Table 6-2.

Most cases of endocapillary proliferative glomerulonephritis appear to result from excessive formation of immune complex. The immune complex is formed by the combination of antigen(s) and antibodies. A wide variety of infectious agents called antigens have been implicated in the pathogenesis of diffuse proliferative glomerulonephritis (or acute glomerulonephritis). These infectious agents (antigens) can be broadly divided into: bacteria, spirochetes, parasites, and virus. Briefly, the responsible organisms are (1) bacteria: gram positive—*Streptococcus hemolyticus, Streptococcus viridans, Staphylococcus aureus, Pneumococcus* species; gram negative—*E. Coli, Pseudomonas;* (2) spirochetes: *Treponema pallidum;* (3) parasites: *Plasmodium, Trichinella spiralis;* (4) virus: myxovirus, varicella, coxsackie B., HAA, mumps, infectious mononucleosis. For details of pathogenesis, see Chapter 4.

The immune complex is characterized by the presence of discrete electron-dense deposits or granular fluorescence of immunoglobulins and complement component(s) in the glomeruli, tubules, or arterial vessels.

Using EM and immunofluorescence microscopy (IFM), proliferative glomerulonephritis can be separated into anti-GBM antibody GN, immune complex GN, and nonspecific type. The distinguishing EM features of anti-GBM antibody GN and immune complex GN are shown in Table 6-3. Since electron-dense deposits may appear alike in acute GN, e.g., diffuse proliferative GN, and in the early stage of persistent (chronic) GN, e.g., membranous or mesangioproliferative GN, distinction must be made between acute and chronic GN on the basis of other ultrastructural findings, such as GBM changes, proliferation of cellular elements, exudation, and mesangial changes. Apart from these, some features of the electron-dense deposits often help to distinguish acute GN from persistent (chronic) GN.

The EM study of glomeruli in diffuse proliferative GN generally reveals the following changes:

1. Increase in number and size of endothelial cells.

Table 6-2
Features of Diffuse Proliferative Glomerulonephritis as Distinguished by Light Microscopy[a]

Groups	Endocapillary proliferation	Exudation of PMN	Crescents	Necrosis of glomerular capillaries	Intracapillary thrombi	Small artery or arteriolar changes
Poststreptococcal GN	+++	+++	Rare	Rare	0	0
Lupus GN	+ to ++ segmental, focal	+ to ++	Sometimes, and no more than 10–20% glomeruli	Often and segmental	+ to ++ (wire loop appearance)	0
Rapidly progressive GN	0 to +	+	+++, 60–95% glomeruli	++ to +++	0	0
GN in acute or subacute bacterial endocarditis	++ focal, segmental	++	Rare	+	0	0
GN in Henoch–Schonlein syndrome	+ to ++	+	+ to ++, 20–40% glomeruli	+ to ++ (sometimes)	0	+ to ++, necrosis, and periarterial inflammatory reaction (sometimes)
Polyarteritis group	+ to ++	+ to ++ (eosinophil <50% glomeruli)	Sometimes	+ to ++ (sometimes)	+ to ++ (sometimes)	+++, necrosis of the arterial wall with periarterial inflammatory reaction
Thrombotic thrombocytopenic purpura	+ to ++	0 to +	Sometimes, <50% glomeruli	+ to ++	+++ most often	+++ arteriolar thrombi

[a] +, Mild; ++, moderate; +++, severe; 0, no change.

Table 6-3
Ultrastructural Differences between Anti-GBM Antibody GN and Immune Complex GN

Glomeruli	Anti-GBM antibody GN	Immune complex GN
Basement membrane	Mild to markedly abnormal. The abnormalities consist of irregularity and spongy appearance on the endothelial side, splitting, and insinuation of PMN through the disruptive basement membrane	In acute immune complex GN, e.g., proliferative GN, basement membrane is essentially normal
Endothelial cell	No change to mild proliferative change	Marked proliferation with occlusion of many capillary loops
Epithelial cell	Pronounced proliferation and adhesion with proliferated Bowman's epithelial cells (crescents)	Slight or no proliferation of epithelial cells and rarely adhesion with Bowman's epithelial cells (uncommon crescents)
Mesangial cell and matrix	Essentially normal	Moderate to marked cellular proliferation and increased matrix in the mesangium may be observed in some types
Electron-dense deposits	Continuous linear electron-dense deposits along the basememt membrane. It is poorly defined and difficult to demonstrate in most cases	Discrete and prominent electron-dense deposits seen on the epithelial aspect or endothelial aspect or both aspects of basement membrane, and within the GBM. Electron-dense deposits also may be seen inside the epithelial cell
Exudation of PMN	Not a pronounced feature	Generally a pronounced feature
Necrosis and collapse of capillaries	Frequent and pronounced	Infrequent and inconspicuous
Fibrin	Often found within glomerular capillary lumina or within crescents	Occasional findings
Tubules	Thickening, irregularity, and disruption of basement membrane and atrophy of cells	Except for the presence of electron-dense deposits within the basement membrane of a few tubules, the latter are generally uninvolved
Interstitium	May be very pronounced due to infiltration by excessive collagen fibers, fibroblasts, lymphocytes, and plasma cells	Generally unremarkable

2. One to two neutrophil leukocytes in each capillary loop. Exudation of this magnitude means about 50 to 100 neutrophil leukocytes per glomerulus. This is highly abnormal, since no more than two to three neutrophil leukocytes are found in the normal glomerulus.

3. A greater number of mesangial cells (generally up to two cells in each mesangium is considered normal).

4. Electron-dense deposits.

5. Generally normal GBM.

6. Necrosis (infrequent) in segment or segments of the glomerulus.

7. A normal number of epithelial cells. Sometimes marked proliferation of epithelial cells leads to crescent formation.

8. Obliteration of Bowman's space by proliferated epithelial cells along with neutrophil leukocytes, fibrin, and/or collagen fibers (crescents).

The associated findings of discrete electron-dense deposits in the tubules, inflammatory cells and collagen fibers in the interstitium, and necrosis and/or electron-dense deposits in the arterioles constitute adjunctive diagnostic features. The degree of exudation of neutrophil leukocytes and the characteristics of electron-dense deposits have been found to be useful indices in the determination of the etiology of endocapillary proliferative GN.

The electron microscopy findings in diffuse proliferative GN can be separated into three broad groups:

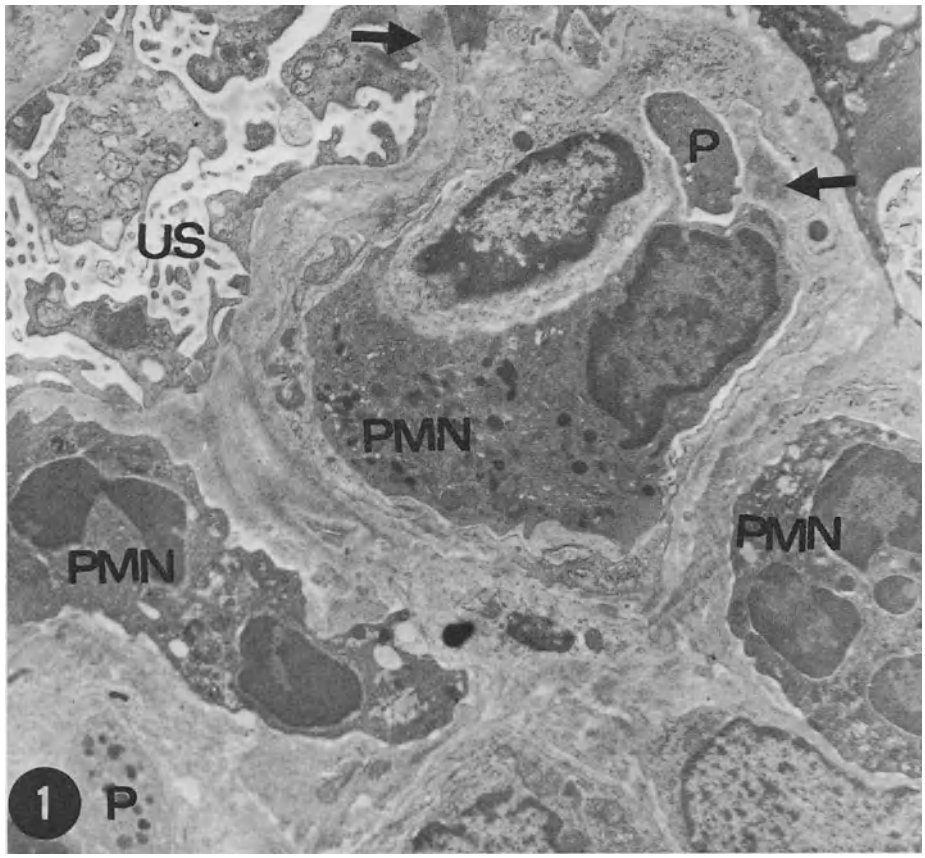


Fig. 6-1. This electron micrograph reveals occlusion of glomerular capillaries by neutrophil leukocytes (PMN). Note platelets (P) inside glomerular capillaries, and small discrete electron-dense deposits (arrows). Swelling of glomerular capillaries is evident from narrowing of urinary space (US) (UA + LC, ×12,000).

Group I. Marked proliferation of endothelial and mesangial cells, marked exudation of PMN, and conspicuously discrete electron-dense deposits, mainly on the epithelial aspect of GBM ("humps"), also within GBM, and rarely on the endothelial aspect of GBM.

Group II. Moderate to marked proliferation of endothelial and mesangial cells, mild exudation of PMN, and very noticeable electron-dense deposits, mainly on the endothelial aspect of GBM and also within the GBM.

Group III. Remarkable proliferation of epithelial cells with slight or no proliferation of endothelial and mesangial cells, mild exudation of PMN, marked disruption of the GBM, and subtle or poorly discernible electron-dense deposits.

Pathologically, groups I and II denote endocapillary proliferative glomerulonephritis, and group III is synonymous with extracapillary proliferative glomerulonephritis.

Group I

The aforementioned glomerular change characterizes poststreptococcal glomerulonephritis (Figs. 6-1 and 6-2). This type of glomerular change, but of lesser

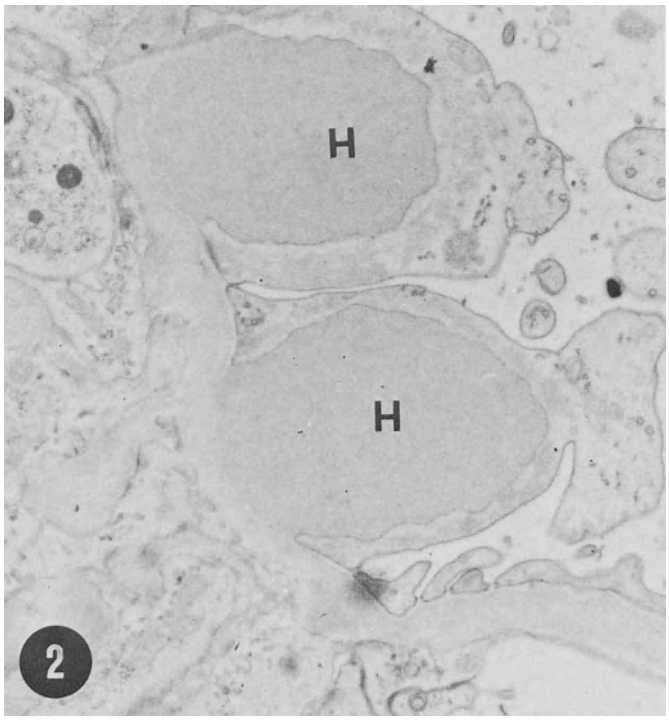


Fig. 6-2. Two adjacent typical "humps" (H) in a glomerular capillary from a patient with poststreptococcal glomerulonephritis. She had elevated ASO titer and low serum C3 (UA + LC, ×15,000).

intensity, has been observed in glomerulonephritis caused by bacterial endocarditis or secondary syphilis (Fig. 6-3). Although the features are more or less alike, the intensity of exudation of PMN and the character of "humps" can distinguish poststreptococcal glomerulonephritis (PSGN) from glomerulonephritis caused by bacterial endocarditis or secondary syphilis (Figs. 6-1 and 6-2 versus Fig. 6-3).

The epithelial or typical "hump" is the most impressive among all the findings in diffuse proliferative GN. Kimmelstiel and associates (1962) were the first to find "humps" in the study of renal tissues from a group of clinically evident PSGN. This finding has subsequently been reported by many investigators. Although initially "hump" was considered to be the pathognomonic feature of PSGN, this view is no longer shared by most individuals. However, experienced observers can distinguish the typical "hump" from the atypical "hump."

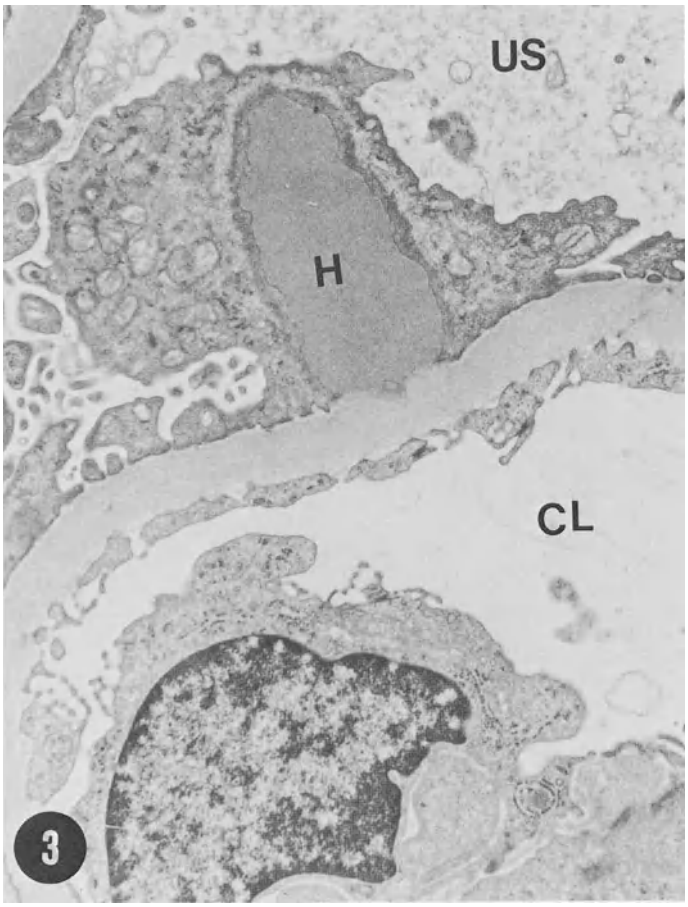


Fig. 6-3. Shows a "hump" which appears like a typical "hump" (H) in a glomerular capillary. Note the absence of limiting membrane in this hump. Lumen of the glomerular capillary (CL) and urinary space (US) are shown. From a patient with acute bacterial (staphylococcal) endocarditis (UA + LC, ×13,000).

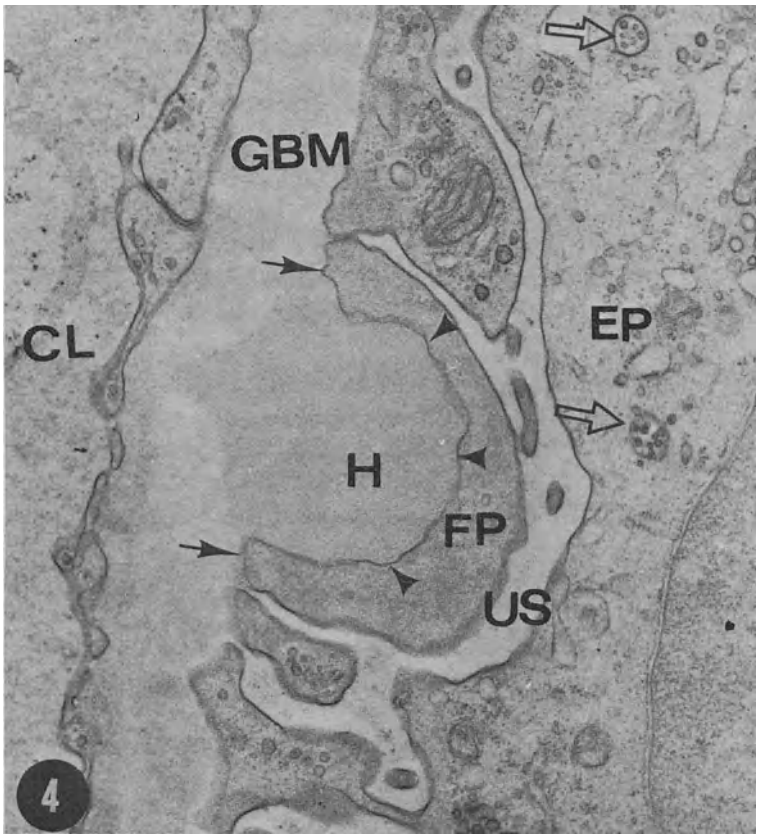
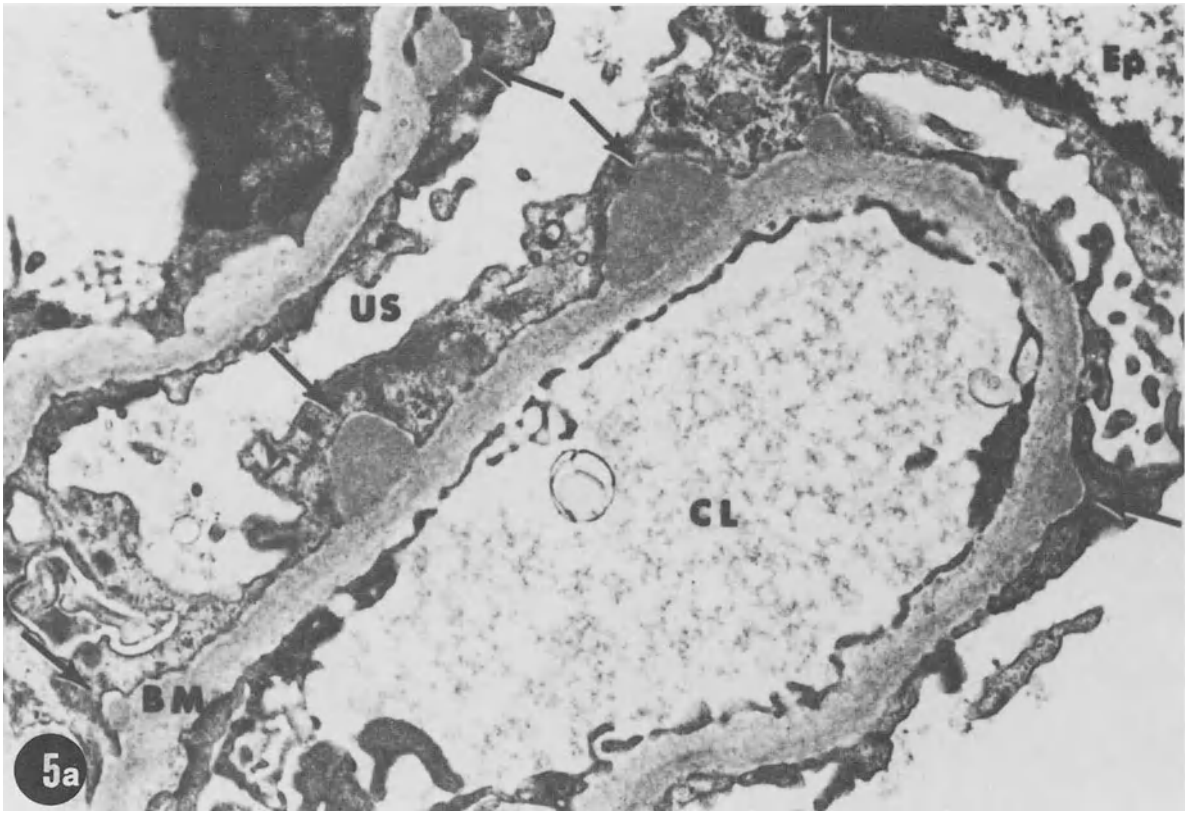


Fig. 6-4. A typical "hump" (H) on the epithelial side of the glomerular basement membrane (GBM). Note the complete limiting membrane (arrowheads) surrounding the "hump" and the continuation of the limiting membrane (arrows) with that of the overlying foot process (FP). Lumen of the glomerular capillary (CL), urinary space (US), and epithelial cell (EP) are shown, along with multivesicular bodies (open arrows) in the epithelial cells (UA + LC, ×20,000).

A typical "hump" (Figs. 6-2 and 6-4) is a circumscriptive electron-dense deposit located on the epithelial aspect of the GBM. It is triangular or ovoid in shape; the base, or one end, rests on the epithelial side of GBM: the apex, or the other end, lies in the urinary space and is covered by an expanded foot process in the form of an umbrella. It has a diameter of 2 to 2.5 μm, occupies the surface area of GBM equivalent to one to two foot processes, exhibits an electron density which is deeper than the intervening segments of the GBM, and is completely surrounded by a dark limiting membrane which is continuous with the membrane of the covering foot processes (Figs. 6-2 and 6-4). The segment of GBM in apposition with the "hump" is slightly expanded and more electron dense than

Fig. 6-5. (a) Note numerous "humps" (arrows) on the epithelial side of the glomerular basement membrane (BM). These humps are regarded as atypical "humps" because of lack of limiting membranes and fusion of foot processes throughout the glomerular capillary. Lumen of the glomerular capillary (CL), urinary space (US), and epithelial cell (Ep) are shown. From a patient with syphilitic glomerulonephritis (UA + LC, × 13,000). (b) A schematic diagram of the appearance of atypical humps (H), which are devoid of limiting membranes. Note continuous fusion of foot processes (FP). Lumen of the capillary (CL) and urinary space (US) are shown.



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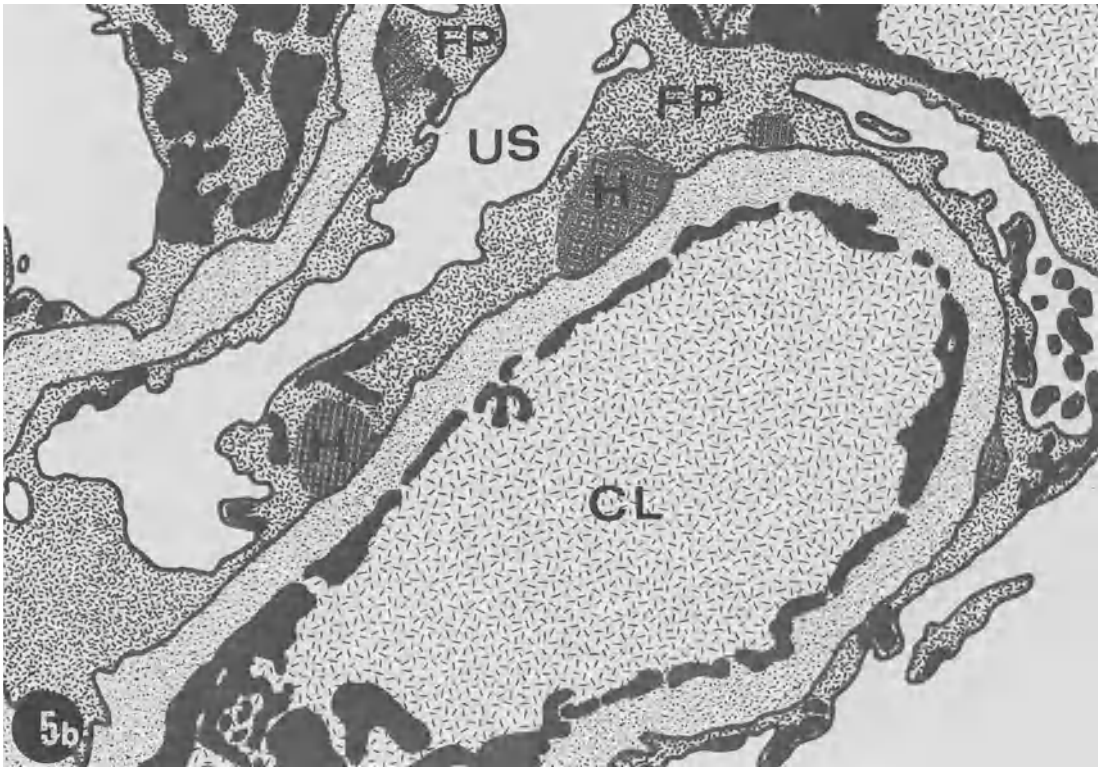


5b

the adjacent segments of the GBM (Fig. 6-4). One to three typical "humps" may be seen in a single capillary loop (Fig. 6-2). It is not uncommon to find a "hump" located at the junctions of the peripheral and centrilobular portions of the capillaries (Fig. 6-2). An atypical "hump" differs from a typical "hump" by the smaller size, shape, partial or complete absence of the limiting membrane, low electron density, and lack of covering by a single foot process (Figs. 6-5a and 6-5b).

Although a typical "hump" is found consistently in poststreptococcal GN, this EM sign alone cannot be regarded as the diagnostic criterion of poststreptococcal glomerulonephritis. This is primarily because a "hump" resembling a typical "hump" has been observed in GN associated with secondary syphilis, bacterial endocarditis, and also occasionally in type I mesangioproliferative GN (Figs. 6-3 and 6-6). The puzzling aspect of this finding is that a typical "hump" has been observed in renal biopsies from patients without clinical or serological evidence of streptococcal infection. In contrast, a typical "hump" was not found

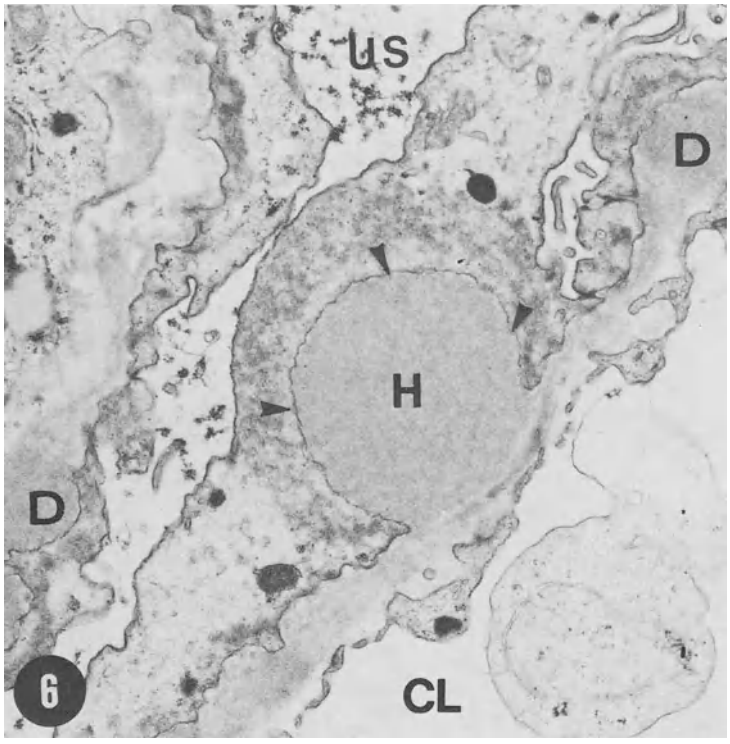


Fig. 6-6. Typical "hump" (H) in the glomerular capillary loop. The limiting membrane of the typical hump (arrowheads) is shown. Also note intramembranous electron-dense deposits (D). Lumen of the capillary (CL) and urinary space (US) are shown. From a patient with type I mesangioproliferative glomerulonephritis (UA + LC, ×14,000).

in the renal biopsies from patients with clinical and serological data supportive of poststreptococcal GN. Some of these ambiguities are exemplified by a patient report (see later).

A typical "hump" can be observed in poststreptococcal GN if the renal biopsy is studied within 1 to 3 weeks after onset of the disease. The appearance of a "hump" becomes less frequent after 4 weeks and even less frequent after 6 weeks. It is not known how these humps disappear, although two possibilities have been suggested: (1) They are pinched off from the GBM and reach the urinary space and finally are excreted in the urine. This is likely since free immune complexes have been isolated from the urine of patients with poststrep-

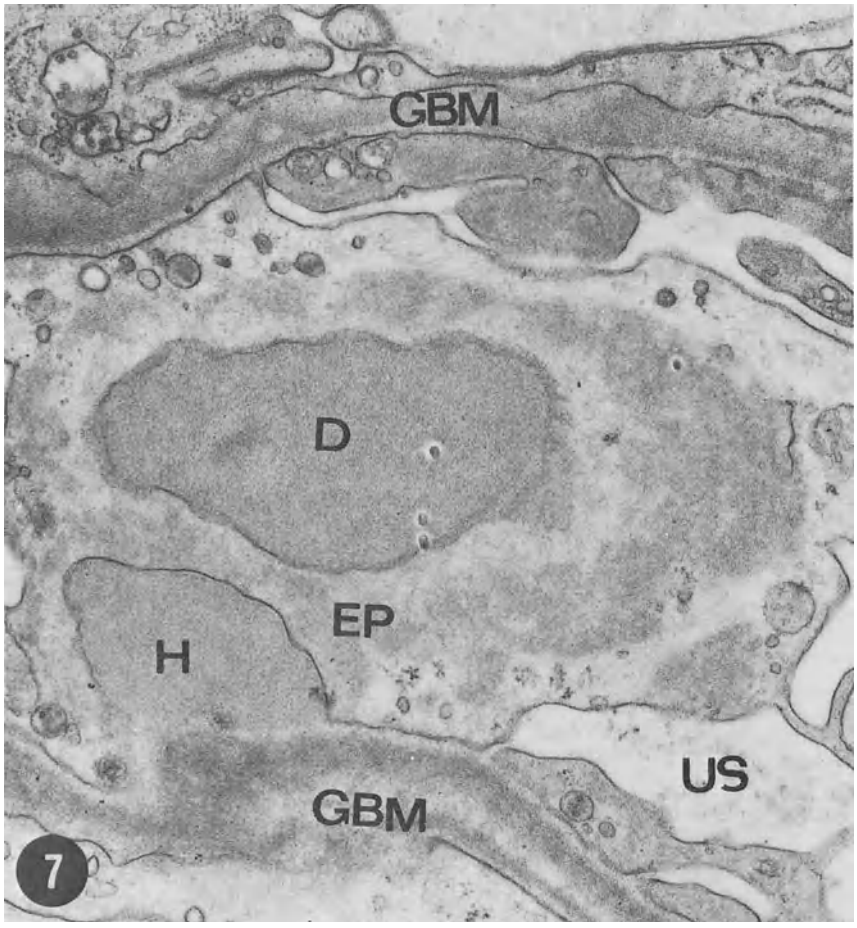


Fig. 6-7. This electron micrograph of the renal biopsy study of a patient with poststreptococcal GN shows a fading "hump" (H) and a "hump"-like deposit (D) within the epithelial cell (EP). Adjacent glomerular basement membrane (GBM) is electron-dense. A typical "hump" from the same biopsy is shown in Fig. 6-4 (UA + LC, ×18,000).

tococcal glomerulonephritis. Our finding of free electron-dense deposits within the epithelial cells along with the presence of "humps" (Fig. 6-7) also supports this pathway. (2) They are phagocytosed by the mesangial cells (the evidence for this is weak).

Patient H.K., an 8-year-old white male, was admitted to Oklahoma Children's Memorial Hospital in June 1975 with complaints of edema of the face and hematuria, which had been preceded by soreness of the skin for 2 weeks. Physical examination revealed blood pressure 180/130 mm Hg and facial edema. Laboratory studies—Urinalysis: 2+ protein, numerous RBC and 1 to 15 WBC/HPF; throat culture normal flora; ASO titer 625 Todd units; BUN (blood serum urea nitrogen) 79 mg/100 ml; serum C3 less than 30 mg/100 ml. Patient was treated with low sodium diet, diuretics, and penicillin. A percutaneous renal biopsy was done and the tissue was studied by LM and EM. LM showed accentuated lobulation of the glomerular tufts, moderate degree of proliferation of epithelial and endothelial cells, marked exudation of neutrophil leukocytes, and moderate to marked increase of mesangial matrix (Fig. 6-8). Loss of tubules and interstitial fibrosis were also

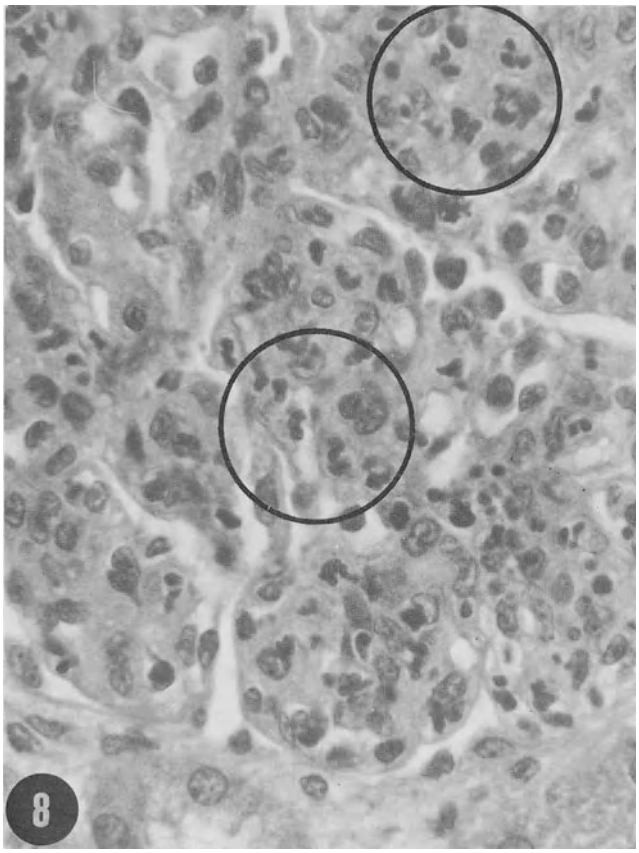


Fig. 6-8. Light micrograph of a glomerulus from a clinically evident poststreptococcal glomerulonephritis. Note accentuated lobulation of the glomerular tufts and excessive exudation of neutrophil leukocytes (circles). The electron micrograph is shown in Fig. 6-1. There is a complete absence of "humps" in this renal biopsy study (H & E, ×320).

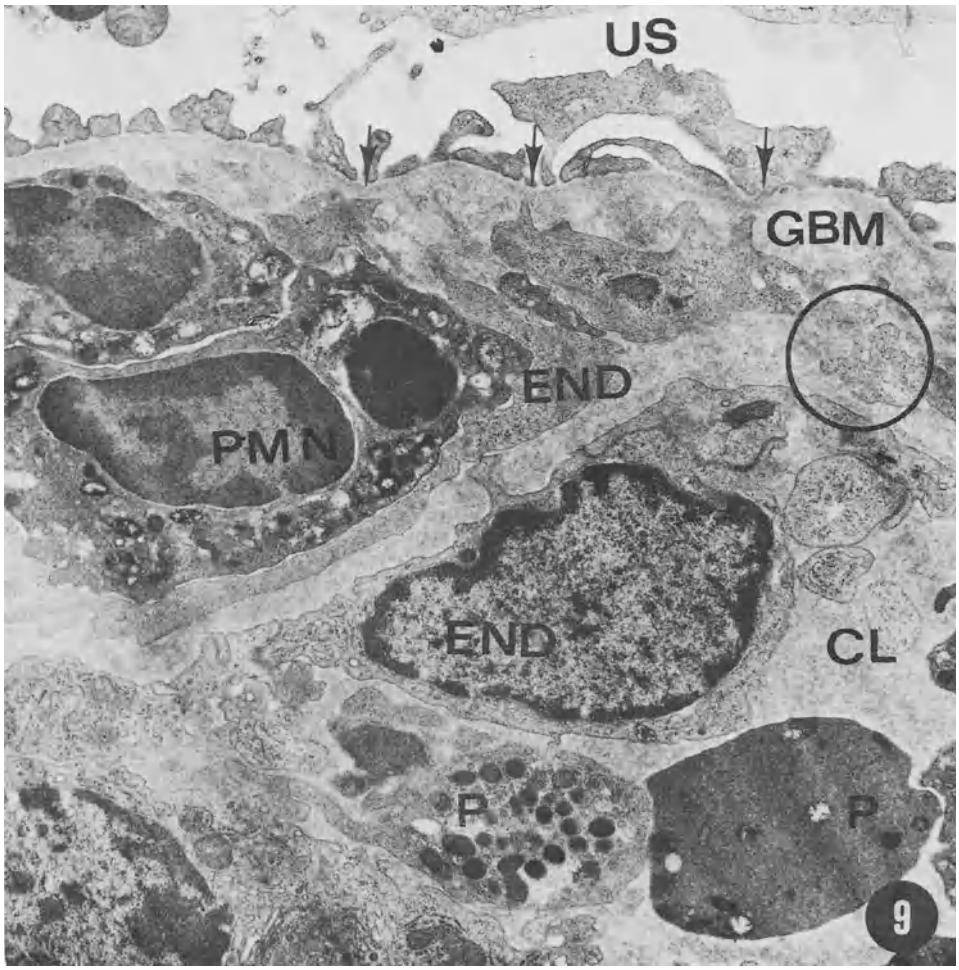


Fig. 6-9. Disruption of the glomerular basement membrane (GBM) characterized by splitting (circle), thinning of the GBM (arrows), and insinuation of a neutrophil leukocyte (PMN) between GBM and endothelial cell (END). Urinary space (US) and an aggregate of platelet (P) within the lumen of the glomerular capillary (CL) are shown (UA + LC, ×18,000).

striking. By EM, glomeruli were characterized by marked destructive changes in the GBM, adhesion of the leukocytes, occlusion of glomerular capillary lumina by excessive numbers of neutrophil, lymphoctye, and sometimes platelet aggregates, and free PMN in Bowman's space (Figs. 6-1, and 6-9 to 6-12).* There were rare and small electron-dense deposits within the GBM but complete absence of a typical "hump." Platelet aggregates with or without fibrin were observed in the peritubular capillaries and veins (Fig. 6-12).

Four weeks after the onset of symptoms, his blood pressure became normal (110/90 mm Hg) and BUN dropped to 26 mg/100 ml; however, microhematuria persisted. After 5 months, he had normal blood pressure (110/70 mm Hg) and normal urinalysis.

* Figures 6-9 through 6-12 show detailed structural changes of the glomerulus shown in Fig. 6-8.

The clinical and laboratory findings in this patient, including serum C3 measurements, were consistent with poststreptococcal glomerulonephritis. The LM study of the renal biopsy supported the clinical diagnosis, but the EM study showing absence of a typical "hump" was inconclusive.

Group II

The ultrastructural features, especially discrete and deep electron-dense deposits located in the subendothelial aspect of GBM, characterize lupus proliferative glomerulonephritis. (See Figs. 6-13 to 6-15 for the active stage of diffuse

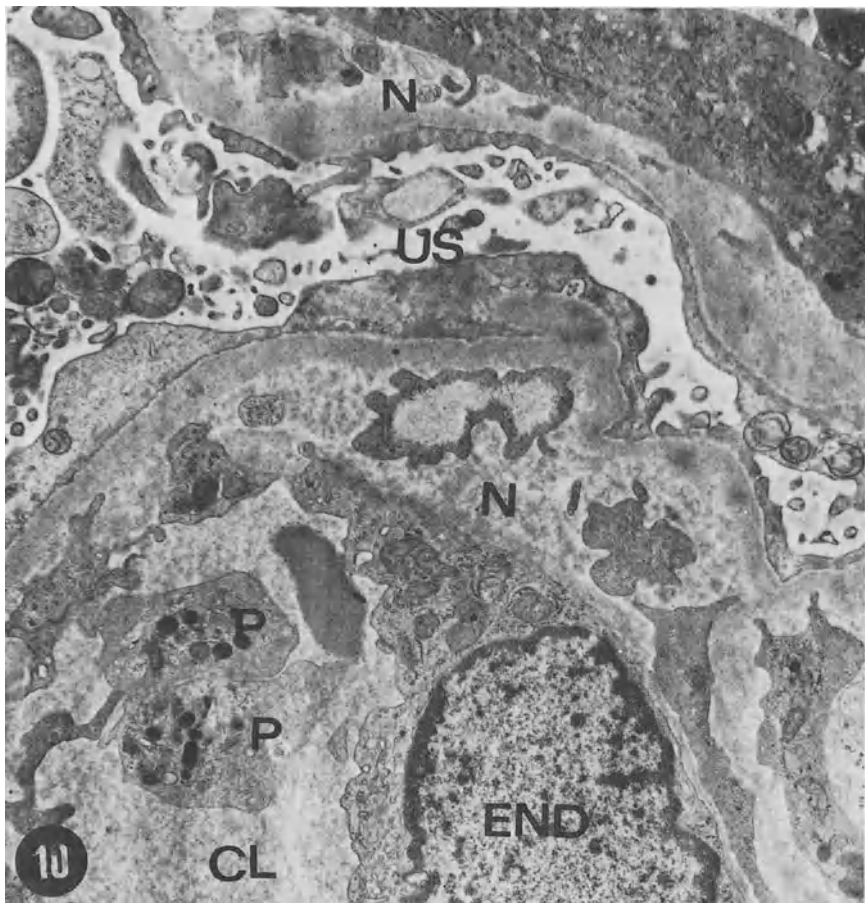


Fig. 6-10. In each of the two adjacent glomerular capillaries a wide electron-lucent zone is seen to intervene between glomerular basement membrane and detached endothelial cell (END). This may be reminiscent of electron-dense deposit or it may represent necrosis (N). The glomerular basement membranes appear to be necrotic. An aggregate of platelets (P) within the lumen of a glomerular capillary (CL) is also seen. Urinary space (US) is shown (UA + LC, ×17,000). .

proliferative lupus glomerulonephritis from patient S. P. Details are given in Chapter 12.) It is the most common form of lupus nephritis seen in biopsy materials. A few small deposits within the GBM and variable amounts of deposits within the mesangium (Fig. 6-15) are common in lupus proliferative GN. The ill-defined LM findings of wire loop lesion and fibrinoid necrosis (Fig. 6-16) have been clarified by EM study; these LM appearances correspond to subendothelial massive electron-dense deposits (Fig. 6-13). Discrete intramembranous deposits, along with extramembranous (epimembranous) deposits, are found frequently in lupus membranous GN (Fig. 6-17) or in membranous transformation of lupus proliferative GN (Fig. 6-18). Caution must be exercised in relying upon the subendothelial deposits alone as a diagnostic criterion for lupus proliferative GN,

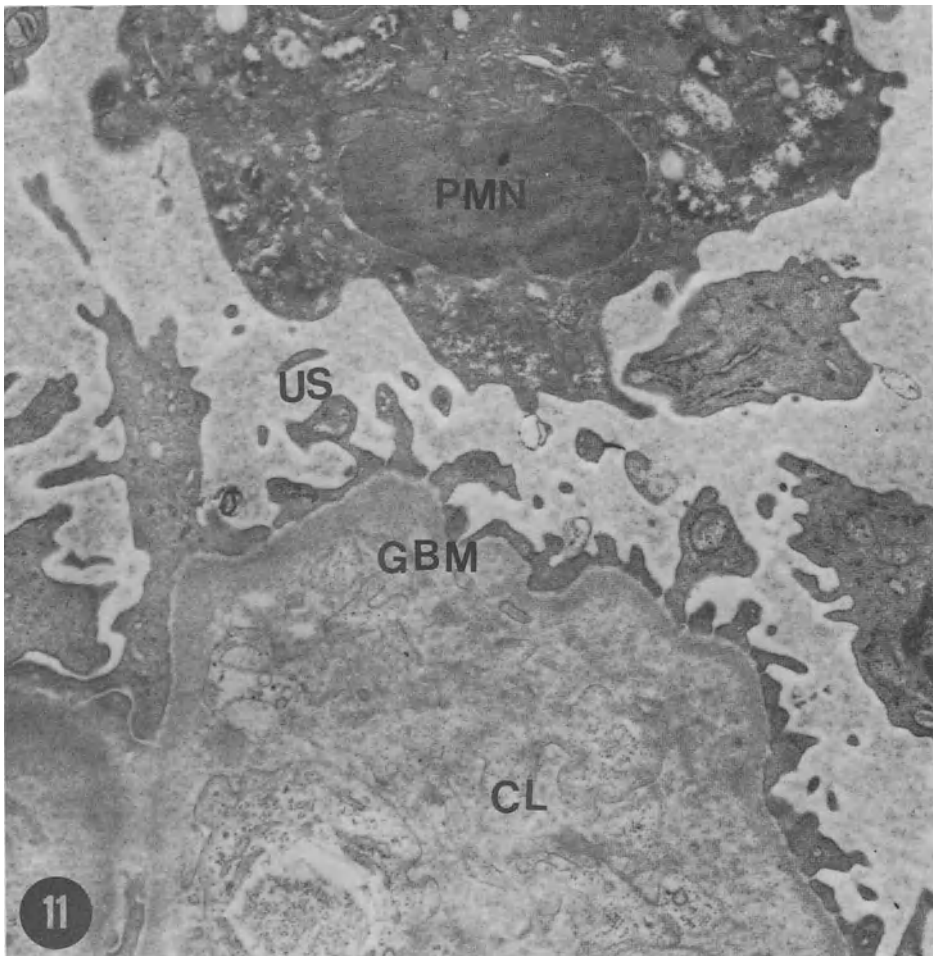


Fig. 6-11. Necrosis of a glomerular capillary. The glomerular basement membrane (GBM) is featureless, i.e., necrotic, and the capillary lumen (CL) is filled with necrotic materials. A free neutrophil leukocyte (PMN) is seen in the urinary space (US) (UA + LC, ×18,000).

because similar deposits have been noted in sclerosing glomerulonephritis, type I mesangioproliferative glomerulonephritis, glomerulonephritis associated with gammopathy, toxemia of pregnancy, hepatic glomerulosclerosis, hemolytic uremic syndrome, and malignant hypertension. In all these conditions, however, unlike lupus proliferative glomerulonephritis, the deposits are small in amount, less widespread, and exhibit low electron density. Sometimes the subendothelial deposits appear to be more organized, with alternate dark and light bands, and resemble "fingerprints" (Fig. 6-14). This appearance, in conjunction with the microtubular structures within the endothelial cells (Fig. 6-19), supports a diagnosis of lupus nephritis.

Lupus nephritis has been classified differently by various investigators. Each of these classifications has its own advantages and disadvantages. I have found

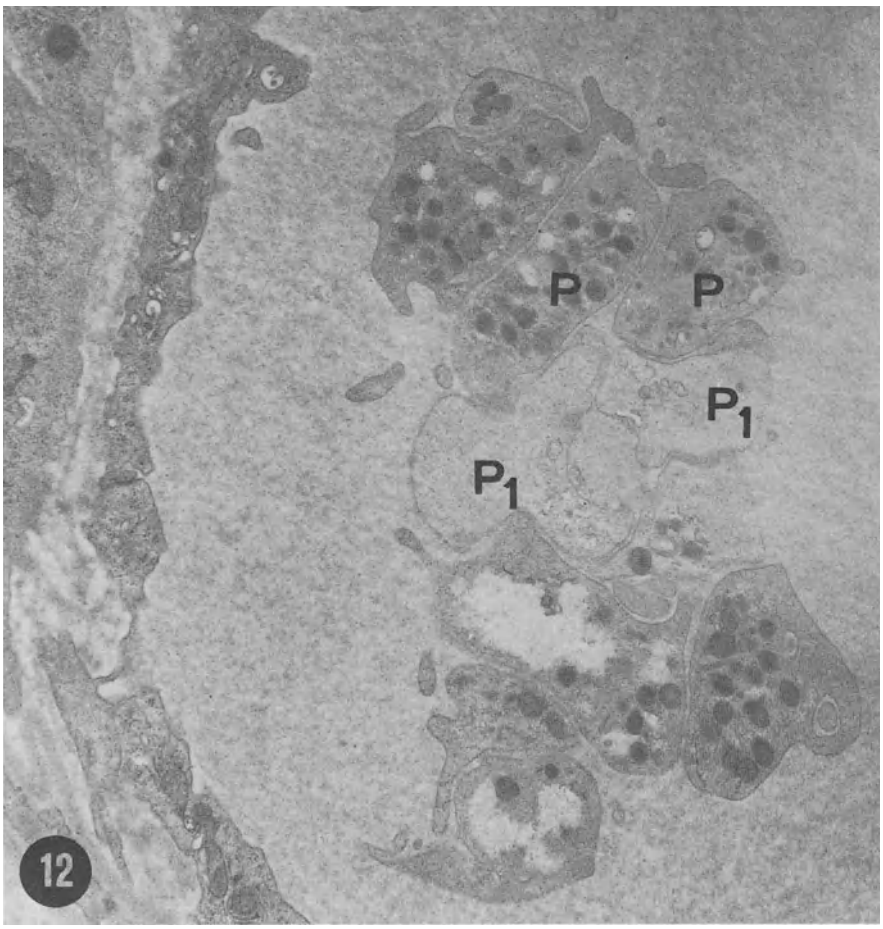


Fig. 6-12. Aggregates of platelets within the lumen of a vein; although some platelets (P) appear normal, others (P_1) are degranulated (UA + LC, ×17,000).

the classification quoted by Dr. McCluskey (1976) (Table 6-4) to be very practical. I believe that this classification would be a helpful guide to physicians in judging the severity of different types of lupus nephritis, as well as in planning appropriate management and defining the course of the disease.

Minimal Lupus Nephritis

Light microscopy in this lupus nephritis is normal or shows slight increase in mesangial cellularity and matrix. EM reveals dense deposits within the mesangium and sometimes along the GBM of the centrilobular portions of the glomerular capillaries. IFM shows mesangial accumulations of IgG and C3.

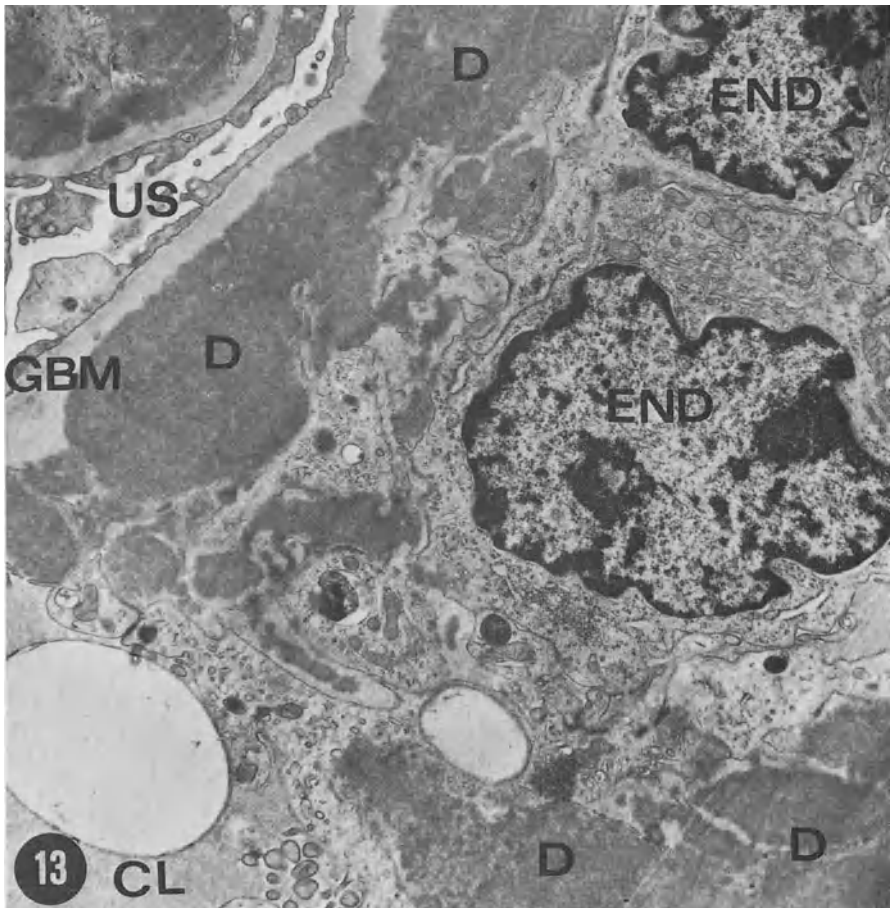


Fig. 6-13. Note the massive amounts of deep electron-dense deposits (D) between the glomerular basement membrane (GBM) and proliferated endothelial cells (END). The capillary lumen (CL) is compromised. Electron-dense deposits (D) are also seen in the mesangium. Similar changes are seen in the glomerular capillary above. Urinary space (US) is shown (UA + LC, ×15,000).

This may manifest in both mild and severe forms, the differences between the two residing in the extent of involvement of individual glomeruli and the percentage of glomeruli involved. Ultrastructurally, the two forms remain indistinguishable.

Membranous Lupus Nephritis

This subject is dealt with in connection with membranous GN.

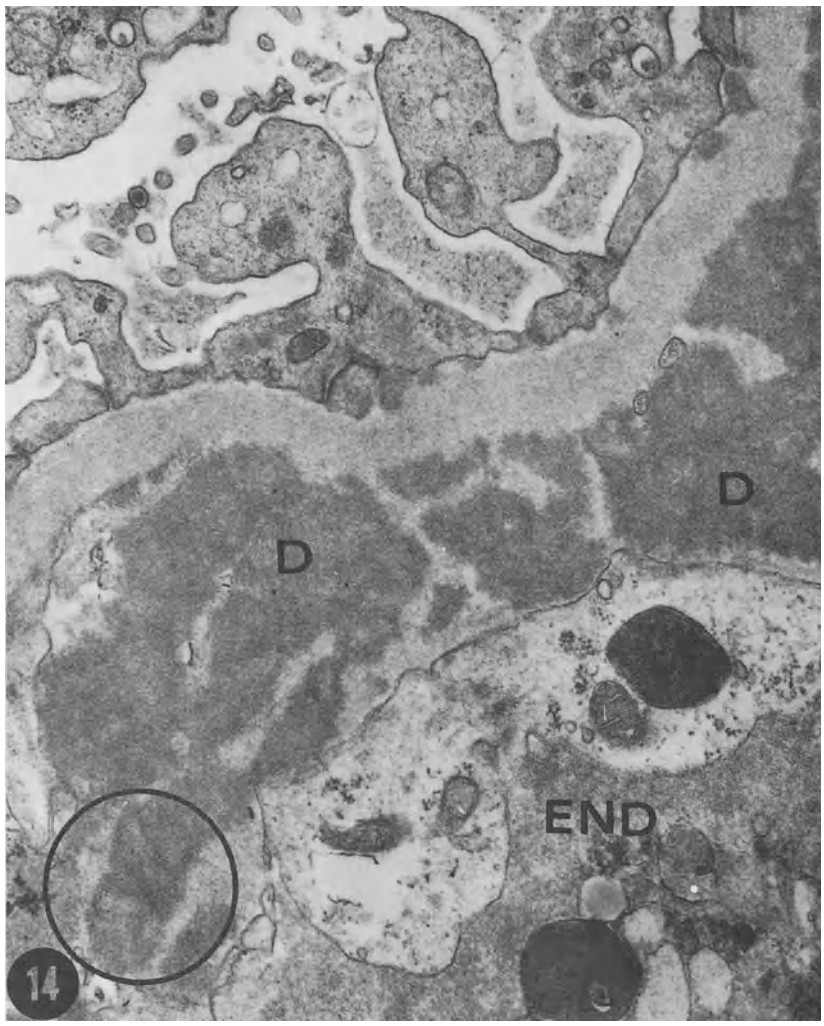


Fig. 6-14. Magnified view of the subendothelial electron-dense deposits (D). Note thumbprint appearance (circle) in the deposits. Endothelial cell (END) can be seen (UA + LC, ×18,000).

Table 6-4
Classification of Lupus Nephritis with Approximate Incidence of Life Expectancy

Classification	Approximate incidence (%)	Approximate survival at 5 yr (%)
Minimal lupus nephritis	30–40	80–90
Mild proliferative lupus nephritis	10–20	80
Severe proliferative lupus nephritis	40–50	30–40
Membranous lupus nephritis	10–15	60–80

Source: Reproduced from McCluskey in *Kidney Pathology Decennial (1966–1975)*, by the kind permission of the author and editor.

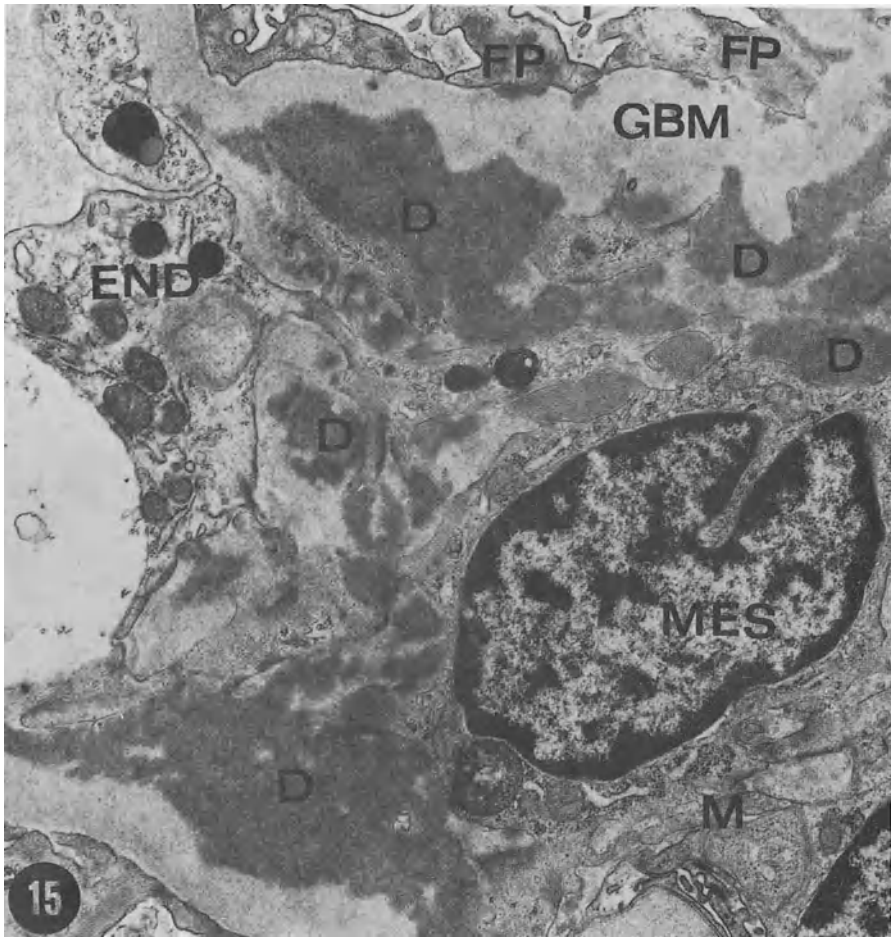


Fig. 6-15. Electron-dense deposits (D) in the centrilobular portion of the glomerular capillaries and in the mesangium (M) are clearly shown. Mesangial cell (MES), vacuolated endothelial cell (END), glomerular basement membrane (GBM) and foot processes (FP) can be seen (UA + LC, ×18,000).

Group III

This group of ultrastructural findings comprises the anatomical diagnosis of a crescentic glomerulonephritis. It is a mistake to attempt to differentiate the causes of crescentic glomerulonephritis based on EM observations alone. Although the precise demonstration of linear electron-dense deposit, along with other findings, supports a diagnosis of rapidly progressive glomerulonephritis (RPGN), the clinician should not rely too heavily on the linear deposits as a distinguishing feature.

Crescenting Glomerulonephritis

Epithelial or fibroepithelial crescents are found in a variety of glomerulonephritides; in some, it may be a predominant and a conspicuous feature; in others, it may be conspicuous but found in a small number of glomeruli; and in still others, it may be an inconspicuous feature. The breakdown of crescentic glomerulonephritis, according to the prevalence and conspicuity of the crescents, is shown in Table 6-5. It is necessary to mention that much confusion exists

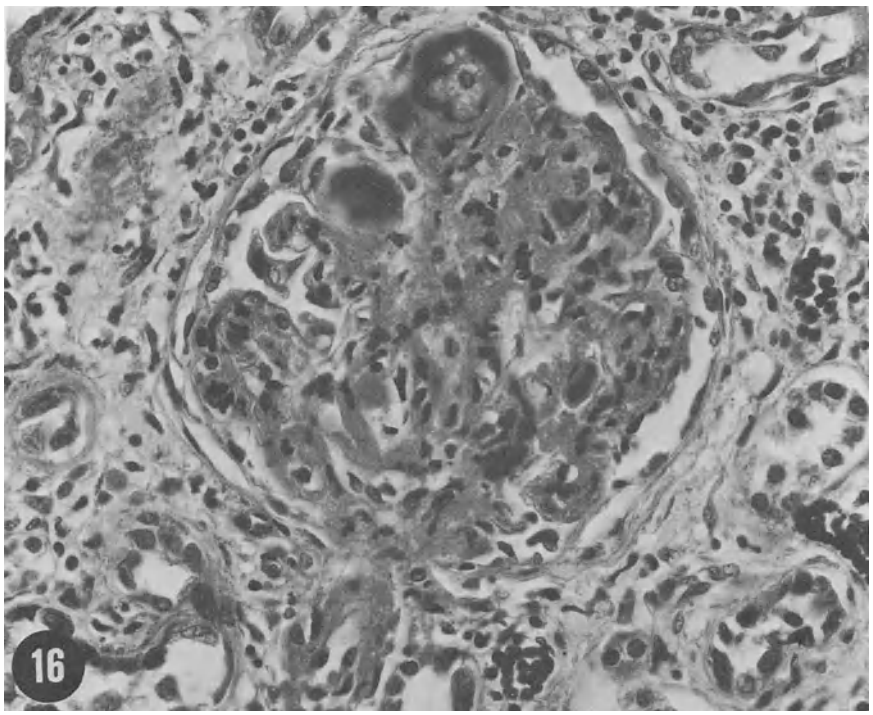
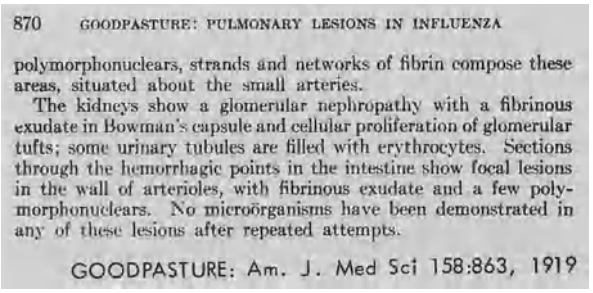


Fig. 6-16. In this light micrograph, dark material on the inner aspect of the glomerular capillaries (described as fibrinoid necrosis), thickening of these glomerular capillaries (wire loop thickening), and hypercellularity in the upper part of the right side of the glomerulus are found (Masson's hematoxylin-phloxine-saffron stain, ×400).

between RPGN and Goodpasture's syndrome. I find it difficult to define Goodpasture's syndrome in view of inadequate information about the renal lesions present in the case with influenza and pneumonia reported by Dr. Goodpasture.

Goodpasture's Syndrome. The renal lesions described by Goodpasture in a single case of severe hemoptysis and right lower lobe pneumonia 3 days after hospital admission on November 6, 1918, are not believed to be consistent with what is now known as rapidly progressive glomerulonephritis. The description of the renal lesion in the reported case is included by direct photography of the portion of the article from the journal in which it was published:



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polymorphonuclears, strands and networks of fibrin compose these areas, situated about the small arteries.

The kidneys show a glomerular nephropathy with a fibrinous exudate in Bowman's capsule and cellular proliferation of glomerular tufts; some urinary tubules are filled with erythrocytes. Sections through the hemorrhagic points in the intestine show focal lesions in the wall of arterioles, with fibrinous exudate and a few polymorphonuclears. No microörganisms have been demonstrated in any of these lesions after repeated attempts.

GOODPASTURE: Am. J. Med Sci 158:863, 1919

After reviewing this article, we have several questions regarding the relationship of the renal lesions reported therein and the current entity of rapidly progressive GN. First, description of the renal lesion in Goodpasture's paper is too inadequate to use in comparison with the vast morphology of RPGN in current practice. In this respect, we are in complete agreement with Dr. J. P. Merrill (1974), of Peter Bent Brigham Hospital and Harvard Medical School, who

Table 6-5
Glomerular Epithelial Proliferation (Crescents) in Renal Disease

Predominant and conspicuous	Less prevalent and less conspicuous	Focal and/or inconspicuous
Rapidly progressive glomerulonephritis	Glomerulonephritis associated with bacteremia or visceral abscesses	Focal proliferative glomerulonephritis
Goodpasture's syndrome	"Hypersensitivity" angiitis	Focal glomerular sclerosis
	Malignant hypertension	Hemolytic uremic syndrome
	Mesangioproliferative glomerulonephritis	Thrombotic thrombocytopenic purpura
	Polyarteritis nodosa	
	Poststreptococcal glomerulonephritis	
	Henoch–Schonlein purpura	
	Systemic lupus erythematosus	
	Wegener's granulomatosis	

Source: Reproduced from Rosen (1975), by the kind permission of the author and editor.

states, "It is questionable whether the syndrome described by Goodpasture is indeed that which bears his name today." Second, there is no evidence to indicate that the mechanism of glomerular lesions described by Goodpasture is anti-GBM antibody which accounts for most cases of rapidly progressive glomerulonephritis. Third, it is not known whether Goodpasture's case had renal failure, whereas rapid and irreversible renal failure is virtually a constant feature in RPGN. Fourth, in glomerulonephritis, hemoptysis might occur as a result of pneumonia or congestive cardiac failure, and yet it bears no relationship to the pathogenesis of glomerulonephritis. Finally, circumstantial evidence of viral illness, e.g., influenza, complicated by glomerulonephritis, might be a helpful guide with which to consider the possibility of Goodpasture's syndrome.

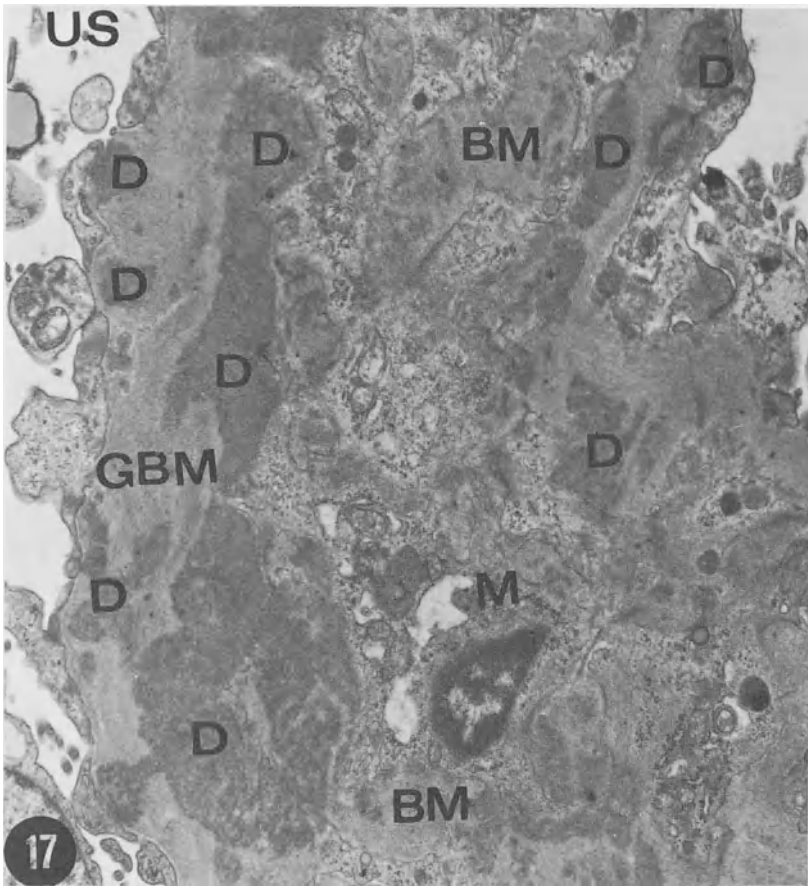

Fig. 6-17. Lupus membranous glomerulonephritis. The electron microscopy demonstrates electron-dense deposits (D) in the subendothelial, intramembranous, and extramembranous locations. The glomerular basement membrane (GBM) is thickened and spiky. There are excessive basement membrane-like (BM) materials and slight proliferative change. Note slight or no deposit in the mesangium (M). Urinary space (US) is shown. The patient had nephrotic syndrome for several years (UA + LC, ×15,000).

This is a clinicopathological entity characterized by a florid crescent in more than 50% of the glomeruli, accompanied by necrosis and/or collapse of glomerular capillaries as well as moderate to marked interstitial change, and almost always associated with rapid and irreversible renal failure. In most cases, there is no identifiable cause which can account for this type of glomerulonephritis; it is therefore called idiopathic rapidly progressive GN (RPGN). Glomerulonephritis occurring in the setting of a variety of conditions may mimic idiopathic RPGN. The differences must be made between idiopathic RPGN and other crescentic glomerulonephritis, and can be achieved preferably and rather easily by light microscopy study (Table 6-2). EM study does not add much to the LM diagnosis.

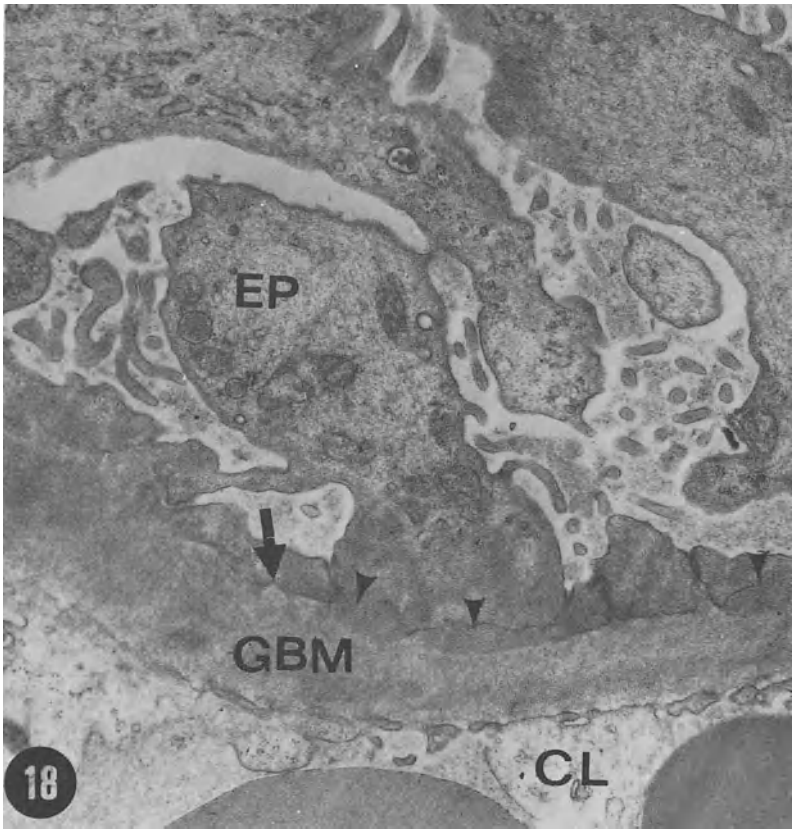

Fig. 6-18. Membranous transformation of lupus proliferative GN. Glomerular basement membrane (GBM) is slightly spiky (arrow) and demonstrates a few extramembranous electron-dense deposits (arrowheads). There is no evidence of proliferative change or subendothelial electron-dense deposits. Capillary lumen (CL) and epithelial cell (EP) are shown. This is the second biopsy from the patient S.P., 1 year after the first biopsy (Figs. 6-13 to 6-15) (UA + LC, ×18,000).

However, EM distinctly demonstrates: (1) absence of endothelial or mesangial cell proliferation, (2) collapse and necrosis of glomerular capillaries, and (3) fibrin in the glomerular capillaries or Bowman's space. IFM is helpful if linear deposits of IgG and C3 can be demonstrated. This is important since management and prognosis differ between idiopathic RPGN and other types of crescentic GN.

Idiopathic RPGN is considered a remorseless renal disease with irresistible progression. A case report is narrated to illustrate this example.

Patient S.B., a 20-year-old black male, was admitted to the University of Oklahoma Hospital on December 21, 1973, with a 3-month history of weight loss, cough productive of blood-tinged sputum, anorexia, fatigue, and fever with occasional chills. Two weeks prior to admission, he experienced progressive weakness and dyspnea on exertion, but no edema. Past history was unremarkable as far as the renal disease was concerned. Physical examination was within normal limits. Blood pressure 136/80 mm Hg. Chest was clear to auscultation. Laboratory studies—Urinalysis: specific gravity 1.016, protein 1+ to 2+, glucose negative, RBC too numerous to count, WBC 35 to 40/HPF, rare granular casts; hemoglobin 8.5 g/100 ml, hematocrit 25 vol %; WBC 7700/mm^3; blood chemistry: serum

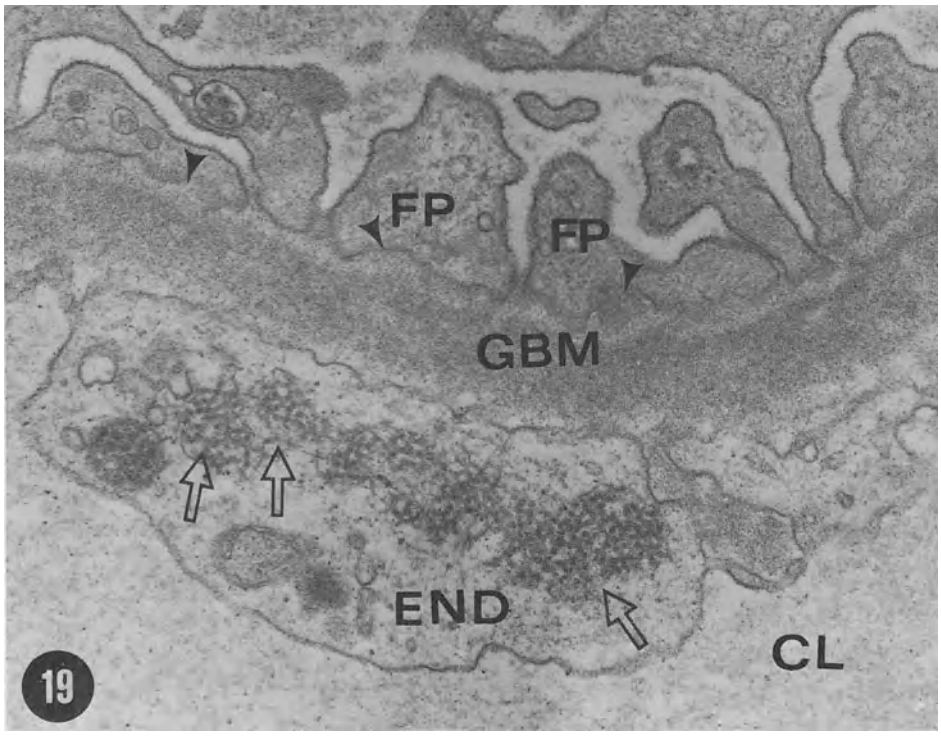

Fig. 6-19. Microtubular structures (viruslike particles) (arrows) within the endothelial cell (END) of a glomerular capillary. The glomerular basement membrane (GBM) is diffusely electron dense and mildly spiky (arrowheads). Discrete foot processes (FP) and lumen of the glomerular capillary (CL) are shown. From a patient with lupus membranous glomerulonephritis (UA + LC, ×22,000).

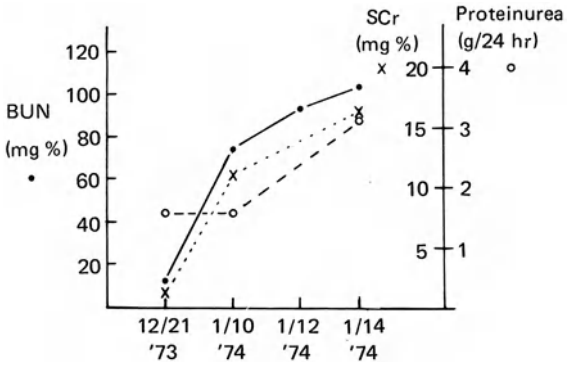

Fig. 6-20. This figure shows the course of progressively severe azotemia and proteinuria in patient S.B., a 20-year-old black male with crescentic glomerulonephritis (idiopathic rapidly progressive glomerulonephritis), demonstrating increased levels of blood urea nitrogen (BUN), serum creatinine (SCr), and proteinuria.

urea nitrogen 12 mg/100 ml, creatinine 1.1 mg/100 ml, 24-hr proteinuria 1.5 g; ASO titer <100 Todd units; serum C3 115 mg/100 ml; chest x-ray: miliary patterns of density throughout both lung fields; x-ray of sinuses normal. He had dramatic rises of serum urea nitrogen, serum creatinine, and urinary protein (which are shown in Fig. 6-20).

A diagnosis of RPGN was made. The history of hemoptysis and miliary densities in the chest x-ray made many consider Goodpasture's syndrome. The patient had an open renal biopsy on January 11, 1974, and the renal tissue was studied by LM, EM, and IFM.

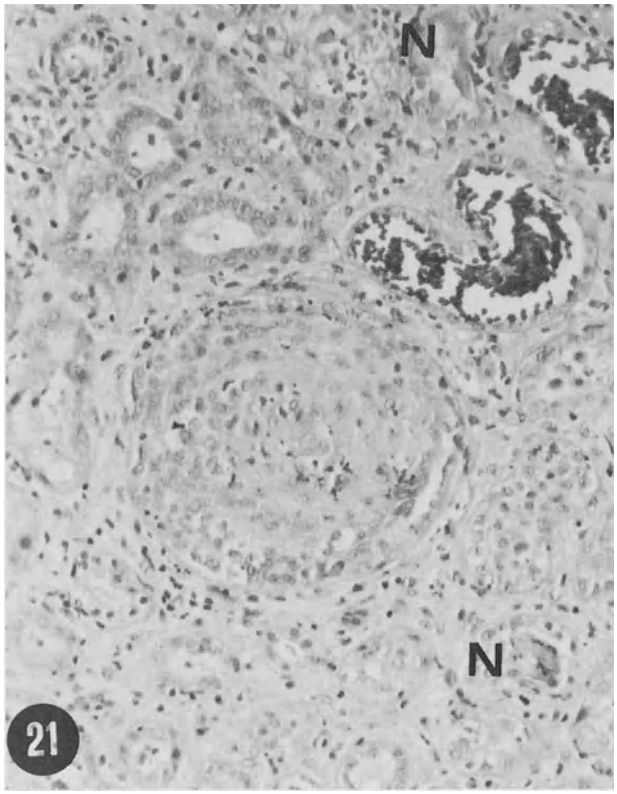

Fig. 6-21. A necrotic and collapsed glomerulus is completely surrounded by a cellular crescent. A few normal tubules are seen (upper left). Two necrotic tubules are filled with blood (upper right), and a few other tubules show necrosis (N). Interstitial fibrosis is evident. From the patient whose course is shown in Fig. 6-20.

LM revealed large crescents in 99% of the glomeruli. The crescents encroached upon two-thirds to three-fourths of each glomerulus with the remaining glomerular capillaries being necrotic or collapsed. The crescents were mostly cellular with a small amount of fibrous tissue. A few tubules were found in the sections, some of which were necrotic and filled with blood. The interstitium was mildly fibrotic (Fig. 6-21). EM revealed disruptive changes in the GBM, spongy electron-lucent appearances reminiscent of linear electron-dense deposit along the endothelial aspect of GBM, exudation of PMN in some glomerular capillaries, attachment of PMN to GBM or even trans-GBM insinuation of PMN, and electron-dense deposit along the endothelial aspect of GBM (Figs. 6-22 and 6-23). There was much edema and collagen fibers observed in the interstitium. The patient was subjected to intermittent hemodialysis but he died 1 month later.

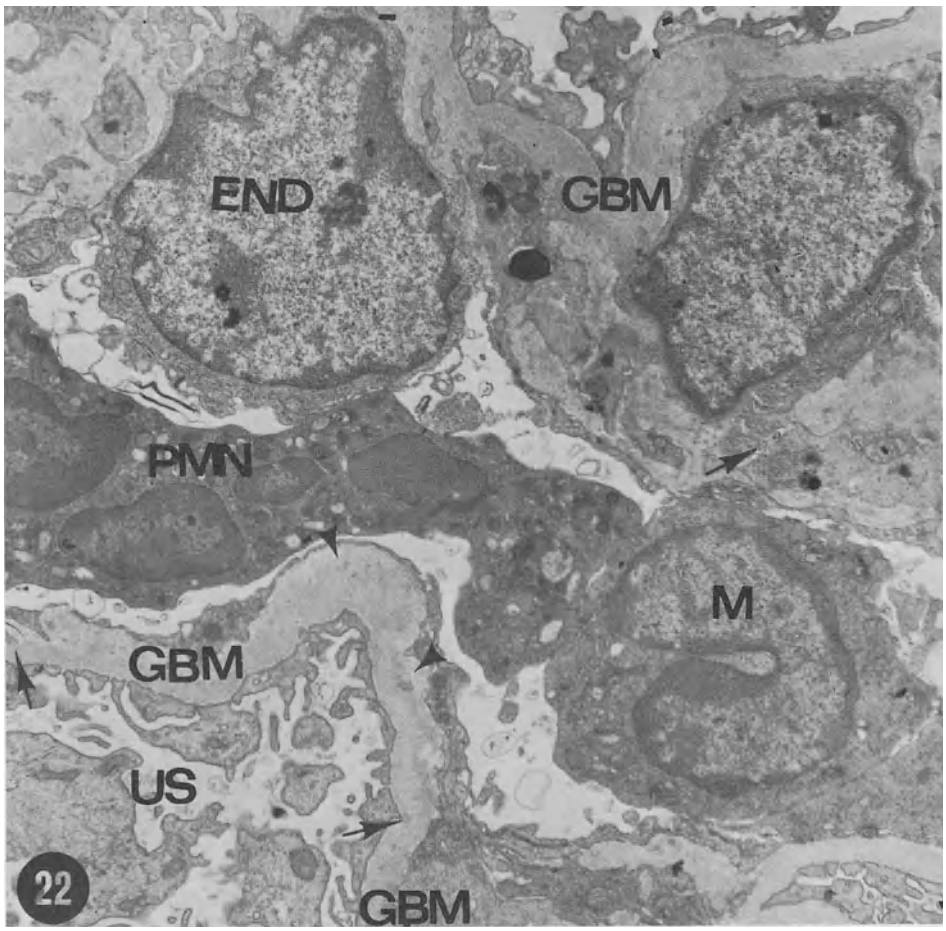

Fig. 6-22. Electron micrograph of a glomerular capillary demonstrates electron lucency linearly along the endothelial aspect of the basement membrane (GBM). This electron-lucent zone (arrowheads) may be reminiscent of a linear electron-dense deposit. Also seen are disruptive changes (arrows) in the GBM and adhesion of a neutrophil leukocyte (PMN) and a monocyte (M) to the endothelial cell (END) and GBM. Urinary space (US) is shown (UA + LC, ×13,000).

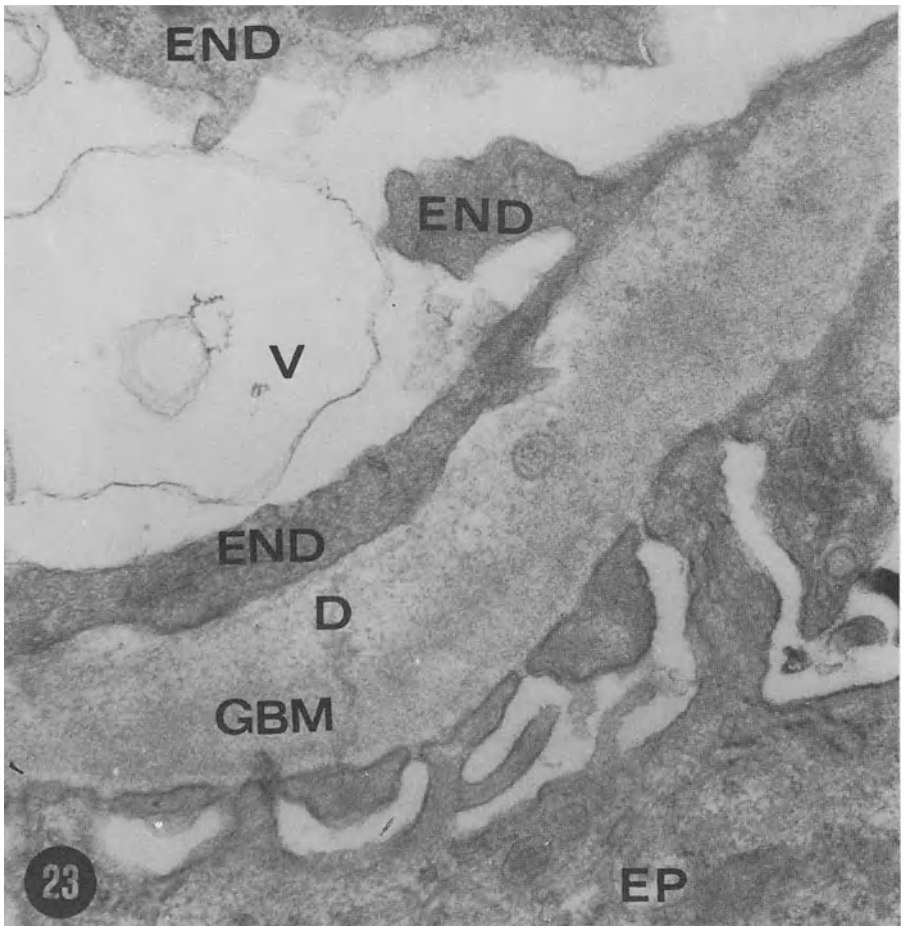

Fig. 6-23. Magnified view of a glomerular capillary clearly demonstrates inner electron-lucent zone which may be reminiscent of deposit or necrosis in the glomerular basement membrane (GBM). Vacuolation (V) and necrosis of endothelial cell (END) and normal appearance of epithelial cell (EP) are shown (UA + LC, ×23,000).

This patient presented with nonspecific symptoms and developed rapid and irreversible renal failure. The renal pathology was that of crescentic glomerulonephritis. Therefore, a pathology of crescentic glomerulonephritis without clinical, serological, or hematological evidence of systemic disease and accompanied by rapid and progressive renal failure is consistent with rapidly progressive glomerulonephritis.

A simulating renal pathology is illustrated in a patient whose presentations were unlike those of the preceding patient.

Patient R.C.,* a 40-year-old white male, was admitted to Lenox Hill Hospital, New

* By the kind permission of Dr. Michael Bruno, Medical Service, Lenox Hill Hospital, New York.

York, with history of multiple nasal bleeding, migratory polyarthralgia, and fever with chills. Admission laboratory tests revealed blood urea nitrogen 75 mg/100 ml, serum creatinine 8 mg/100 ml, 24-hr urinary protein 6.3 g, negative LE cell preparation, and antinuclear antibody (ANA). Chest x-ray revealed parenchymal infiltrations in both lung bases. In addition, there was a small nodular density in the right costrophenic angle. He had an open renal biopsy and the renal tissue was studied by LM, EM, and IFM. Biopsy of the nasal mucosa showed granuloma consistent with Wegener's granulomatosis. He was treated with prednisone and cyclophosphamide, which resulted in some improvement in his clinical picture and renal function.

LM study of renal biopsy revealed lesions in all glomeruli, characterized by crescent encroaching two-thirds to three-fourths of a glomerulus and involving 50 to 60% of the glomeruli. Necrosis of parts of or whole glomeruli, periglomerular cellular infiltration, few dilated tubules filled with blood and necrosis in the arterial vessels were observed (see Figs. 6-24 to 6-26, which are from a patient with Wegener's granulomatosis). EM study

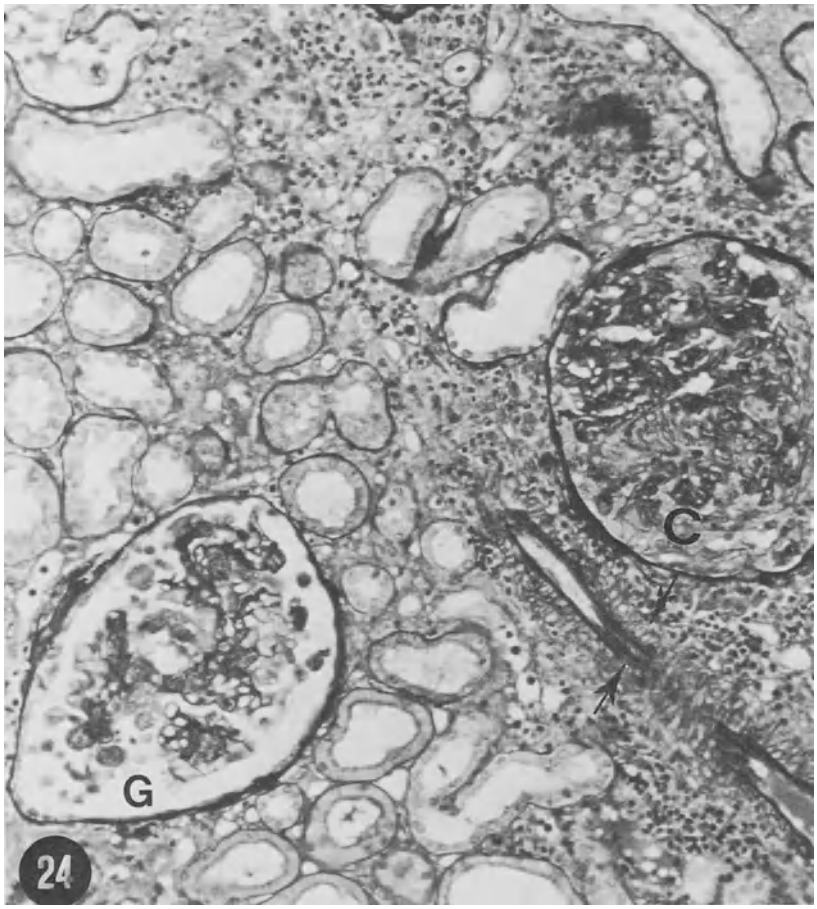

Fig. 6-24. Complete necrosis of glomerular tufts (G), necrosis of capillaries and crescent formation (C) in another glomerulus, and cellular infiltration around the crescentic glomerulus and a small vein (opposing arrows) are shown. Tubules are plentiful and intact (Masson's hematoxylin-phloxine-saffron, ×120).

confirmed the LM findings. No fibrin was found within glomerular capillaries or within the crescent. Interstitium revealed edema and cellular infiltrates (Fig. 6-27a).

The renal pathology observed by LM and EM simulates that of RPGN. The additional features of necrosis of parts of or whole glomeruli and necrosis of the arterial vessels indicated necrotizing glomerulonephritis. The clinical features,

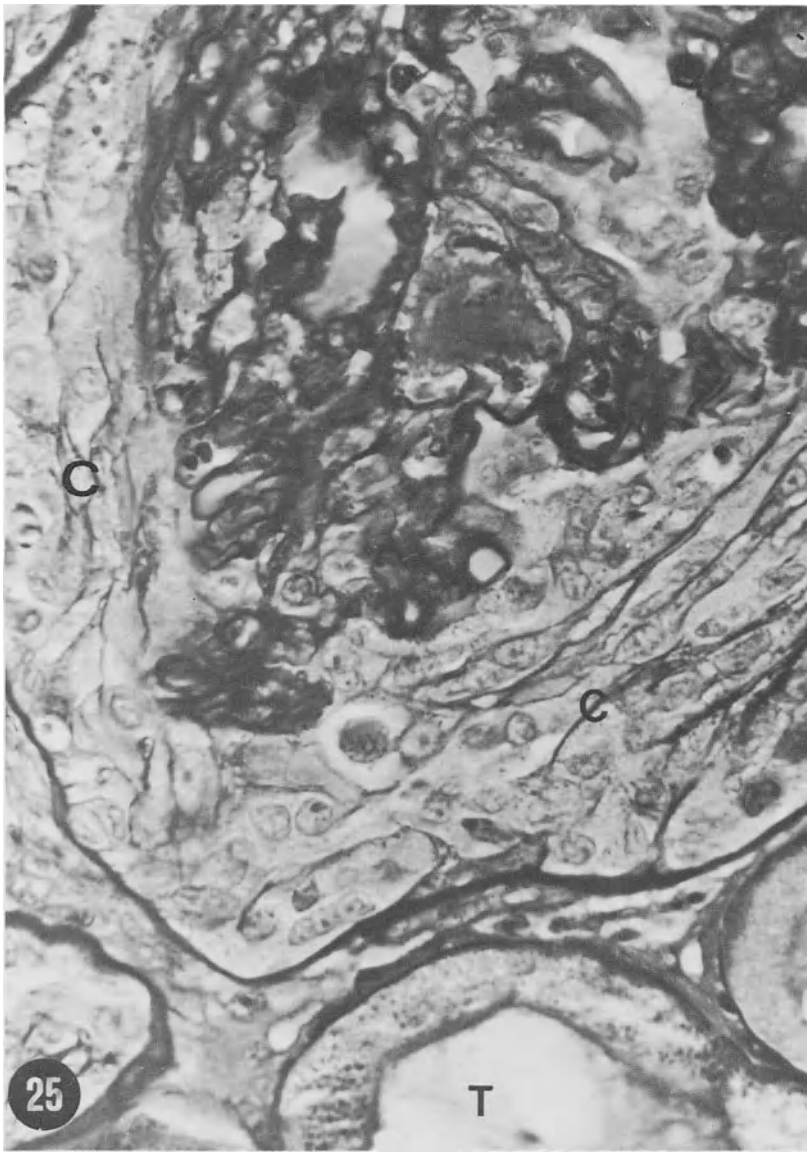

Fig. 6-25. An epithelial crescent (C) surrounds necrotic glomerular capillaries. Tubules (T) appear to be intact. The crescentic epithelial cells resemble tubular epithelial cells. This finding supports the idea that glomerulotubular communication may be an important avenue for crescent formation. For more information, see Mandal *et al.* (1977).

along with the pathology of extracapillary proliferative and necrotizing glomerulonephritis, are consistent with Wegener's granulomatosis. Caution must be exercised not to confuse this renal lesion with that in polyarteritis nodosa. In the latter, arterial vessels show florid periarterial inflammation in addition to necrotic changes.

These clinical examples illustrate that a combination of clinical profiles and renal pathology, and not renal pathology alone, is helpful in the differential diagnosis of crescentic glomerulonephritis.

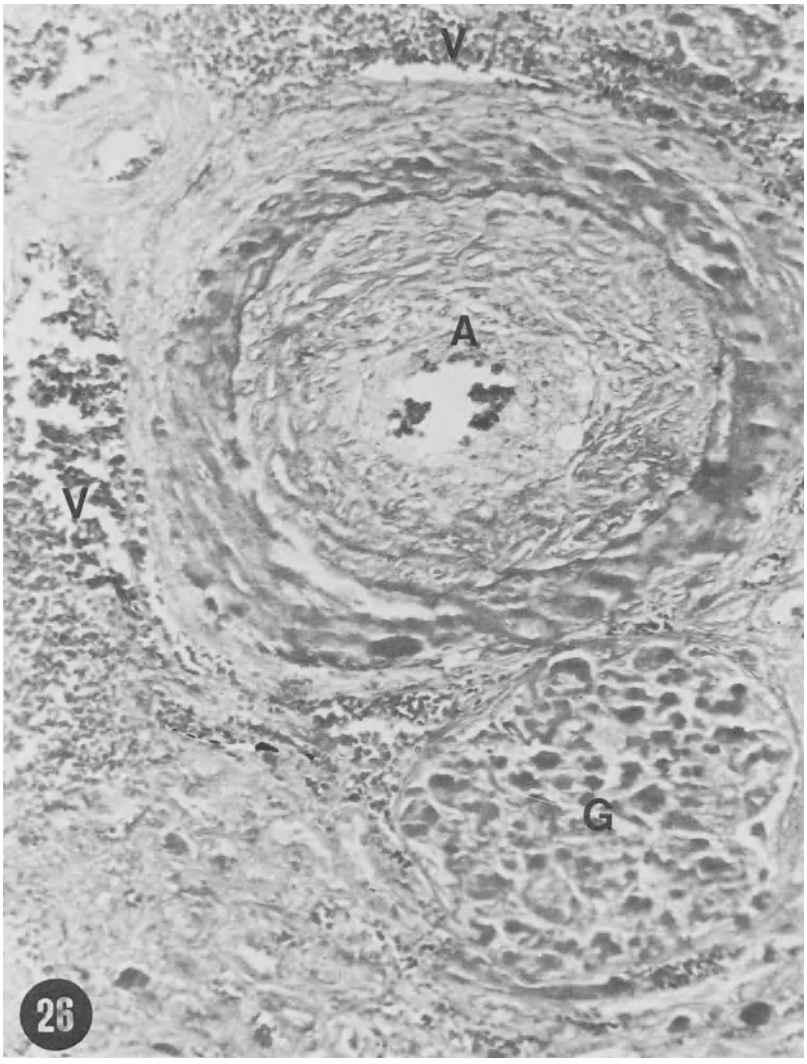

Fig. 6-26. Coagulative necrosis in a glomerulus (G) and in a small artery (A), and hemorrhagic necrosis in a vein (V) are seen (Masson's hematoxylin-phloxine-saffron, ×400).

Syphilitic Glomerulonephritis

Since most of the patients reported had nephrotic syndrome, this topic is described in the chapter on nephrotic syndrome.

Malarial Glomerulonephritis

Although malarial glomerulonephritis is rare in most countries, it appears to be quite common in certain parts of Africa. Since the vast majority of patients with malarial glomerulonephritis present with nephrotic syndrome, this subject is elaborated in the chapter on nephrotic syndrome.

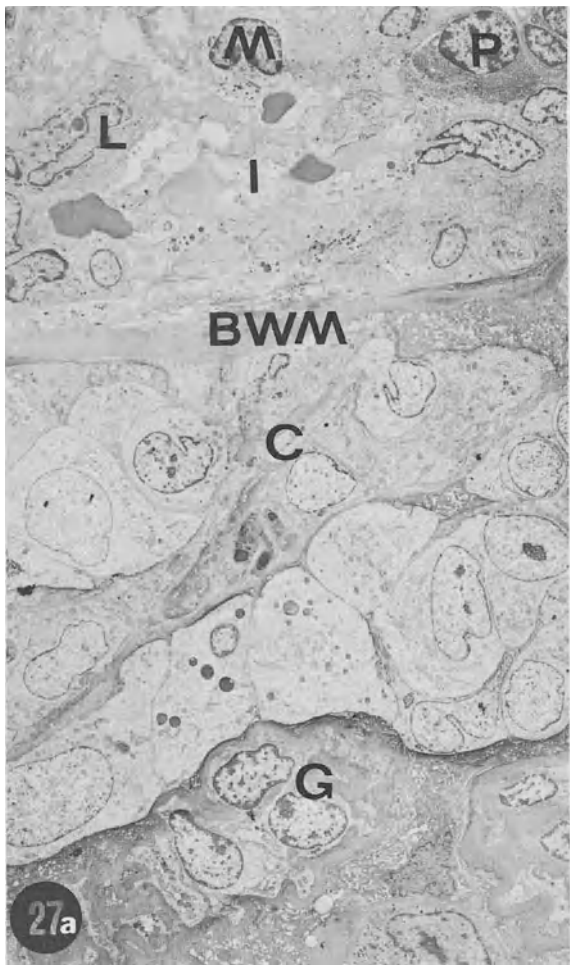

Fig. 6-27a. An electron micrograph reveals marked proliferation of parietal epithelial cells with crescent formation (C) between collapsed glomerular capillaries (G) above and Bowman's membrane (BWM) below; periglomerular infiltration by lymphoctye (L), monocyte (M), plasma cell (P), and collagen fibers in the interstium (I) are observed (UA + LC, ×3000).

The renal pathology may be consistent with diffuse proliferative glomerulonephritis, focal and segmental proliferative glomerulonephritis, or necrotizing glomerulonephritis. By LM study, the pathology may resemble that of poststreptococcal glomerulonephritis, lupus glomerulonephritis, or rapidly progressive glomerulonephritis. Necrotic changes in the small arteries or arterioles may raise the question of hypersensitivity reaction (anaphylactoid purpura). Even so, the necrotizing glomerulonephritis associated with necrotizing arteriolitis resembles renal lesions in Wegener's granulomatosis.

It is reasonable to suggest that in the assessment of acute glomerulonephritis, the possibility of Henoch–Schonlein syndrome should be kept in mind in the differential diagnosis. A combination of preceding history of sore throat and mildly elevated ASO titer, preceding history of insect bite, or history of allergic reactions to food or drug further increases the suspicion of this syndrome, which is more common in children than in adults. The keys to the diagnosis are the clinical setting of the syndrome, such as joint pain, purpuric skin rash, abdominal pain, bloody diarrhea, and normal serum C3 complement (see Table 3-2). For the interest of the reader, the autopsy study of Olser's (1904) case of the erythema group of skin diseases accompanied by manifestations of renal disease (subsequently recognized as anaphylactoid purpura or Henoch–Schonlein syndrome) is included. Dr. W. McCallum did the autopsy of one of the cases (erythema group of skin diseases) of William Osler. He stated

> The glomeruli form the most striking feature in the section. The Malpighian tuft in almost every one is much compressed by the new growth of a mass of cells in the area of the capsular space which formed a crescentic mass. These cells lie in a connective tissue network, which is continuous with the connective tissue outside the capsule. They often have small, subdivided, capsular spaces lined by capsular epithelium, suggesting that the original capsular space was merely invaded by this new growth.

In this connection, I should mention that the vivid description of glomerular crescents made by Dr. McCallum 77 years ago has not changed a bit.

The pathogenesis of GN in Henoch–Schonlein syndrome is very unclear; there is evidence to suggest that the Henoch–Schonlein syndrome, acute nephritis, rheumatic fever, and polyarteritis nodosa together form a family of diseases, linked by the tendency of one member to coexist with another, and by the characteristics common to the group. The pathogenesis common to the members of this group is probably an antigen–antibody reaction similar to that occurring in anaphylaxis and taking place especially in the endothelium of blood vessels. The most common antigen appears to be a derivative of the hemolytic streptococcus, although proteins derived from other organisms or from nonbacterial sources are considered as additional antigens. The target blood vessels involved in this process are the small vessels of the skin in Henoch–Schonlein syndrome, glomerular capillaries in acute nephritis, and the medium-sized and small arteries in polyarteritis nodosa. Although there is much evidence in terms

of the clinical and pathological pattern of this family of conditions to link them with anaphylactic phenomena, the failure to demonstrate the antigen concerned constitutes a bar to our understanding of that group of diseases which is believed to result from bacterial hypersensitivity.

Thrombotic Thrombocytopenic Purpura

The essential pathology in this condition comprises occlusion of the microcirculatory bed. The clinical manifestations are attributed largely to occlusive changes in the capillary beds of organs, in particular, kidney and brain. A combination of hemolytic anemia, thrombocytopenia, and rapidly developing renal failure with oliguria or anuria is a common presentation.

The renal lesions are characterized by the presence of eosinophilic amorphous material (thrombi) in the afferent arteriole which extends into the glomerular capillaries. Mild to marked proliferation of glomerular cellular elements with or without crescent formation may be found. Segmental or total glomerular necrosis or even cortical necrosis is not infrequent. The light microscopy appearance simulates lupus glomerulonephritis, glomerulonephritis of essential cryoglobulinemia, and hyalinosis of focal glomerular sclerosis. If necrosis and proliferation predominate, then this condition must be distinguished from lupus proliferative GN, Wegener's granulomatosis, and renal lesions of severe hypertension. EM study helps merely to confirm the LM findings. Therefore, the diagnosis of TTP is to be established through the process of exclusion with the aid of clinical and laboratory data (see Chapter 3).

The mechanism of the disease process is unclear. Because of the findings of endothelial cell proliferation and electron-dense deposits in the subendothelial aspect of small arterioles, some investigators thought that the primary event was vascular damage with secondary platelet adhesion, aggregation, and subsequent thrombosis.

The etiology of this irreversible pathophysiological condition is still unknown. Instances of its occurrence in siblings or marital partners have implicated environmental causative agents such as virus, bacteria, and toxic agents, which may interact with genetically predisposed hosts.

Hemolytic Uremic Syndrome*

The hemolytic uremic syndrome is considered to be an acute and potentially lethal disease, the final outcome of which is related to the immediate consequences of the renal and extrarenal vascular and capillary thrombotic lesions. The renal pathology resembles that of TTP except that the severity of the lesion may be less than that of TTP. The available data suggest that a significant number

* Reproduced from the original version of Gianantonio *et al.* (1968), by the kind permission of Dr. Gianantonio.

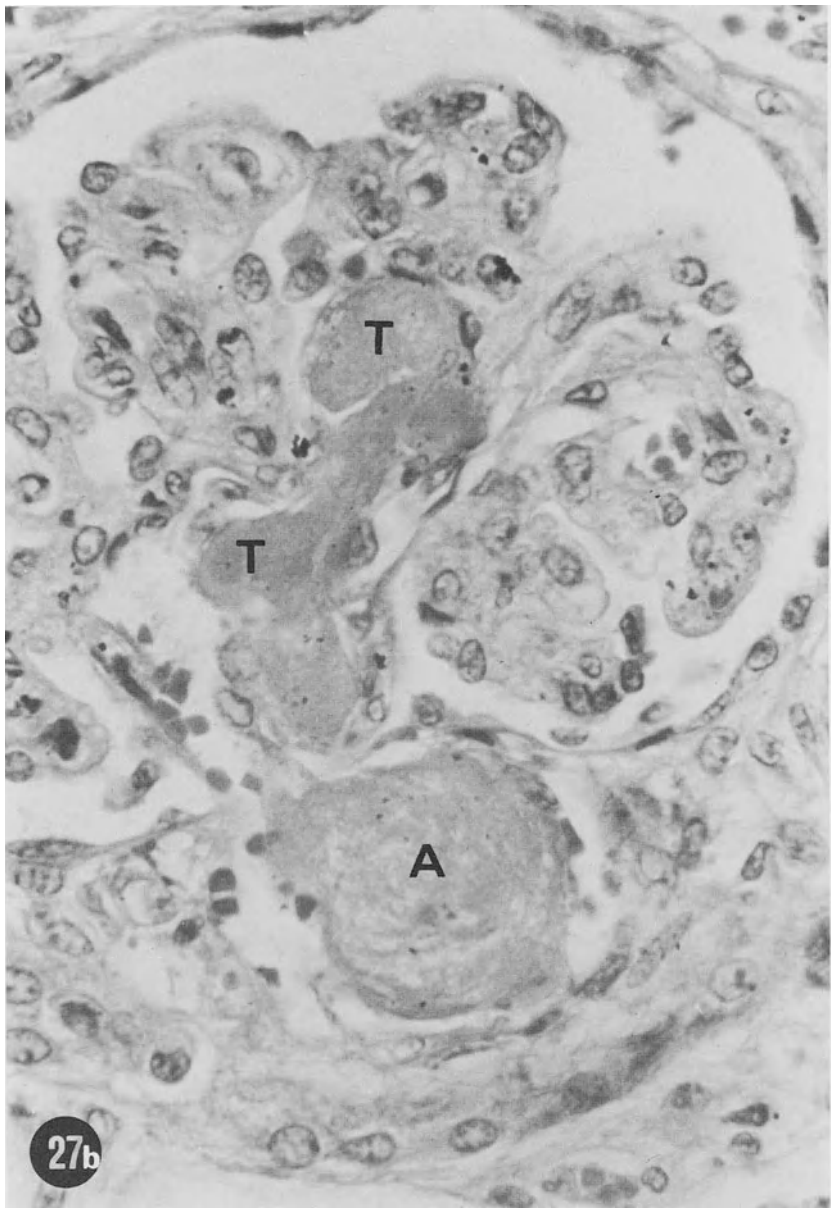

Fig. 6-27b. The glomerulus reveals filling of some capillary loops along vascular axis by a homogeneous eosinophilic material. This material appears to be a thrombus (T). Note necrosis and thrombosis of the afferent arteriole (A). In the entire section one third of the total number of glomeruli reveals this change (H & E, ×320). From a three-year-old white female with hemolytic uremic syndrome.

of the survivors either develop progressive renal disease or are left with severe nonprogressive renal sequelae.

Although the number of renal biopsies studied was rather small and only a single specimen was studied from most of the children, a good correlation was established between the pathological findings and the clinical course.

Twenty-four biopsies from 22 patients were examined and the renal lesions were classified into three types:

Type 1. Cicatricial sequelae form the active stage of the disease, consisting of hyalinized glomeruli, areas of infarction in the hyalinized glomeruli, tubular atrophy, and foci of interstitial fibrosis.

Type 2. Thickening and hypercellularity of the centrilobular areas of the glomerular tufts were observed, which were focal and never severe. These were regarded as resolving or quiescent lesions.

Type 3. Active lesions consisted of epithelial crescents, areas of fibrinoid degeneration in the glomerular tufts and afferent arterioles, severe focal intracapillary proliferation involving even the peripheral capillary loops, segmental thickening of the capillary basement membrane, and interstitial infiltration with lymphoid cells.

Follow-up studies have revealed an increase in incidence of hypertension. The endothelial damage and deposition of fibrin in the arterioles explain the development of acute phase and the sequelae, which when severe are the end result of incomplete cortical necrosis. Nephrotic syndrome has not been observed.

The mechanism of the disease process is unclear. This acute or chronic normocomplementemic glomerulonephritis is apparently a nonimmunological phenomenon. There is clear evidence that a hypercoagulable state leading to fibrin deposition in the glomerular capillaries and the small vessels of the kidney and other organs is a notable feature of the hemolytic uremic syndrome. Serial coagulation studies performed during the acute phase in 27 patients in one reported series provide strong support to the proposed pathogenetic mechanism.

Hemolytic uremic syndrome (HUS) appears to have a geographic predilection, but thrombotic thrombocytopenic purpura does not. Most investigators believe that these two conditions are histologically indistinguishable (Fig. 27b). For the purpose of clarity, the similarities and dissimilarities of the two conditions are shown in Table 6-6.

ASSESSMENTS OF THE ACTIVITIES OF THE PATHOLOGICAL PROCESS IN ENDOCAPILLARY PROLIFERATIVE GN (CLINICOPATHOLOGICAL CORRELATION)

The activities of the pathological process in glomerulonephritis can be evaluated directly by serial studies of the renal tissues, indirectly by laboratory tests,

or preferably by both. There is very little evidence to indicate that histological evaluation alone is superior to laboratory tests in assessment of the activity of the disease process. There are several reports on the serial renal biopsy studies in endocapillary proliferative glomerulonephritis. From the available information in the literature and my own experience, it can be stated that there is a poor correlation between the severity of the glomerular changes and the clinical recovery. This has been exemplified in a patient, H.K. (described earlier). This patient with poststreptococcal glomerulonephritis showed a most severe histopathological picture, which was associated with necrotic and thrombotic changes. Necrosis and thrombosis are rare in poststreptococcal glomerulonephritis, and, according to many observers, they imply a poor prognosis. The rapid recovery and normal follow-up findings in this patient are contrary to this view. Therefore,

Table 6-6
Similarities and Dissimilarities of TTP and HUS

	TTP	HUS
Entity	A syndrome	A syndrome
Age group	Usually adult	Usually infants and young children
Geographic predilection	None	Appears to be common in Latin America
Sex	M:F ratio almost equal	M:F ratio almost equal
Features	Pertains to hemolytic anemia, thrombocytopenia, and renal failure. Neurologic findings may be overwhelming	Same as TTP except neurologic manifestations are less common. Renal failure predominates
Essential pathology	Thrombotic occlusion of small arterial vessels and capillaries throughout the body	Same as TTP
Renal pathology	Variable. Thrombi in the afferent arteriole, glomerular capillaries, glomerular necrosis, crescentic glomerulonephritis	Same as TTP
Laboratory tests	Except thrombocytopenia, no laboratory evidence of intravascular coagulation. Negative antiglobulin test	Elevated levels of factors V and VIII as well as fibrinogen–fibrin split products have been found. Negative antiglobulin test
Course	Usually fatal in days or weeks. Small percentage survive, but become victims of chronic uremia	Much better than TTP, 5% mortality during acute stage, half of the survivors develop chronic uremia
Value of medical therapy	Dubious. Effectiveness of any therapy, e.g., heparin, corticosteroids, and splenectomy, is controversial	Dubious. Anticoagulants
Mechanism(s)	Primary vascular damage vs. intravascular coagulation	Perhaps same as TTP. Hypercoagulable state (Gianantonio *et al.*, 1968) is a distinct possibility

this study provides an example showing that severe pathological changes do not always indicate poor prognosis.

Because excessive endothelial cellular proliferation, PMN exudation, electron-dense deposits, and mesangial hypercellularity are considered important indices of active pathological processes, most or all of the features are absent in follow-up biopsies of the patients who recover from acute glomerulonephritis. The clinicopathological studies in acute glomerulonephritis reported by Hinglais and associates (1974) are stimulating. These workers separated the clinical presentations according to the presence or absence of "humps" and of other glomerular lesions. These are listed in Table 6-7.

The information in the table clearly demonstrates that glomerulonephritis associated with "humps" or with other glomerular lesions has a higher incidence of severe clinical course such as anuria or congestive cardiac failure than glomerulonephritis without "humps." There appears to be no difference in clinical severity between glomerulonephritis with "humps" and those with other glomerular lesions. If anuria is believed to be an ominous sign, this would mean that cases of acute glomerulonephritis associated with "humps" or other glomerular lesions are more sinister types than those without "humps." This is further supported by the lower recovery rates of the patients with "humps" or other glomerular lesions. Patients demonstrating other glomerular lesions, e.g., necrosis

Table 6-7
Initial Clinical Features (65 Cases) of Endocapillary Glomerulonephritis[a]

Features	With humps (N = 35)	Without humps (N = 15)	Other glomerular lesions (N = 15)
Proteinuria >2 g/day	35	15	15
Hematuria	35	15	15
Renal failure	30	10	15
Hypertension	24	9	8
Edema	25	11	12
Oliguria	18	6	8
Anuria	6	0	3
Nephrotic syndrome	11	2	8
Congestive heart failure	4	0	0
Epileptic seizures	4	2	1
Preceding infection			
Upper respiratory tract	29	9	10
Other	2	2	2
Antistreptolysin 0 titer (Todd units)			
>400	16	5	2
>1000	9	2	1

[a] Membranoproliferative glomerulonephritis excluded.
Source: Excerpted from Hinglais *et al.* (1974), by the kind permission of N. Hinglais, M.D., Necker Hospital, Paris, France, and the editor of the *American Journal of Medicine*.

and thrombosis, generally have a tendency toward a worse prognosis (Table 6-8). This study also indicates that patients with typical "humps" have a greater chance of complete recovery than those with atypical "humps" and excessive mesangial hypercellularity. Failure of the patients with atypical "humps" to recover was manifested by significantly higher incidence of nephrotic syndrome, hypertension, chronic renal failure, and increased percentage of glomeruli with sclerosis.

In brief, the healing stage of endocapillary proliferative glomerulonephritis, e.g., poststreptococcal glomerulonephritis, is characterized by (1) decrease in endothelial and mesangial cellularity, (2) decrease in number of PMN, (3) disappearance of "humps," (4) opening of capillary lumina, and (5) normalization of serum C3. Slight mesangial hypercellularity may persist for an indefinite period and does not necessarily mean progressive disease.

The unremitting or progressive endocapillary proliferative glomerulonephritis is characterized by (1) persistent mesangial hypercellularity, (2) increase in mesangial matrix, (3) irregularity of the GBM on the endothelial aspect or spikes on the epithelial aspect, (4) atypical "humps," and (5) persistently low serum C3. It is open to question as to whether unremitting endocapillary proliferative GN is a variant of mesangioproliferative GN (hypocomplementemic glomerulonephritis).

For evaluation of activity of lupus proliferative glomerulonephritis in particular, serial laboratory tests are more useful than serial histopathological studies (see Chapter 3). However, complete studies of the renal tissue, using LM, EM, and IFM, have helped in judging the activity of the pathological process. The histopathological differences between active and inactive lupus proliferative glomerulonephritis are listed in Table 6-9. Some believe that the locations of electron-dense deposits relative to GBM are reliable indices of activity of the disease process. The degree of severity of clinical manifestations relating to this histological feature has been enumerated by Grishman and colleagues (1973) (Table 6-10).

The clinicopathological correlation shown in Table 6-10 documents that se-

Table 6-8
Long-Term Follow-Up (65 Cases) of Endocapillary Glomerulonephritis[a]

	With humps		Without humps		Other glomerular lesions		
	No.	%	No.	%	No.	%	Total
Recovery	23	77	10	100	1	8	34
No recovery	6	20	0	0	9	69	15
Death	1	3	0	0	3	23	4
Lost to follow-up	5	—	5	—	2	—	12
Total	35		15		15		65

[a] Mesangioproliferative glomerulonephritis excluded.
Source: Excerpted from Hinglais *et al.* (1974), by the kind permission of N. Hinglais, M.D., Necker Hospital, Paris, France, and the editor of the *American Journal of Medicine*.

Table 6-9
Histopathological Differences between Active and Inactive Lupus Proliferative GN

	Active lesions	Inactive lesions
Glomeruli	Segmental or diffuse necrosis	Absent necrosis
	Segmental or diffuse hypercellularity and exudation	Normal cellularity
	Massive subendothelial deposits	Intramembranous and extramembranous deposits, a few spikes in the glomerular basement membrane
	Mesangial deposits	Excessive mesangial matrix
	Epithelial crescents	Fibrotic or fibroepithelial crescents
Tubules	Electron-dense deposits in the basement membrane	Thickened basement membrane
Interstitium	Exudation of inflammatory cells and electron-dense deposits	Increase in fibroblasts and collagen fibers

vere clinical manifestation such as renal failure is associated more often with subendothelial electron-dense deposits than with deposits elsewhere in the glomeruli.

Of all the histopathological features just cited, necrosis of glomerular capillaries and crescent formation in more than 50% of glomeruli, regardless of the type of glomerulonephritis, generally indicate an irreversible lesion and a poor prognosis. Most cases of glomerulonephritis complicating the various clinical conditions listed at the beginning of the section on diffuse endocapillary proliferative glomerulonephritis resemble either poststreptococcal glomerulonephritis or lupus glomerulonephritis. These must be distinguished by the clinical profiles and the adjunctive laboratory tests and not by histopathological studies alone (see Chapter 3).

Table 6-10
Site of EM Deposits in Relation to Clinical Manifestations

Site of EM	Number of cases	Renal disease				Outcome: renal failure
		None	Mild proteinuria	Severe proteinuria	Nephrotic syndrome	
None	10	3	5	1	1	0
Mesangial	6	2	3	1	0	1
Subepithelial and mesangial	7	0	0	4	3	4
Subendothelial, subepithelial, and mesangial	8	0	1	5	2	7

Source: Reproduced from Grishman *et al.* (1973), by the kind permission of E. Grishman, M.D., and the editors of *Nephron*.

Membranous glomerulonephritis is characterized by the following changes in the glomeruli: (1) uniform and diffuse thickening of GBM of peripheral capillary loops of all glomeruli (light microscopy), (2) spiky appearance of GBM, and (3) extramembranous (epimembranous) electron-dense deposits. Two of these three criteria must be present to denote a glomerular pathology consistent with membranous glomerulonephritis.

Light microscopy study of paraffin-embedded sections stained with PAS or PAMS is quite useful in furnishing a diagnosis of membranous glomerulonephritis, especially in the advanced stage (Fig. 6-28). However, there are limitations of

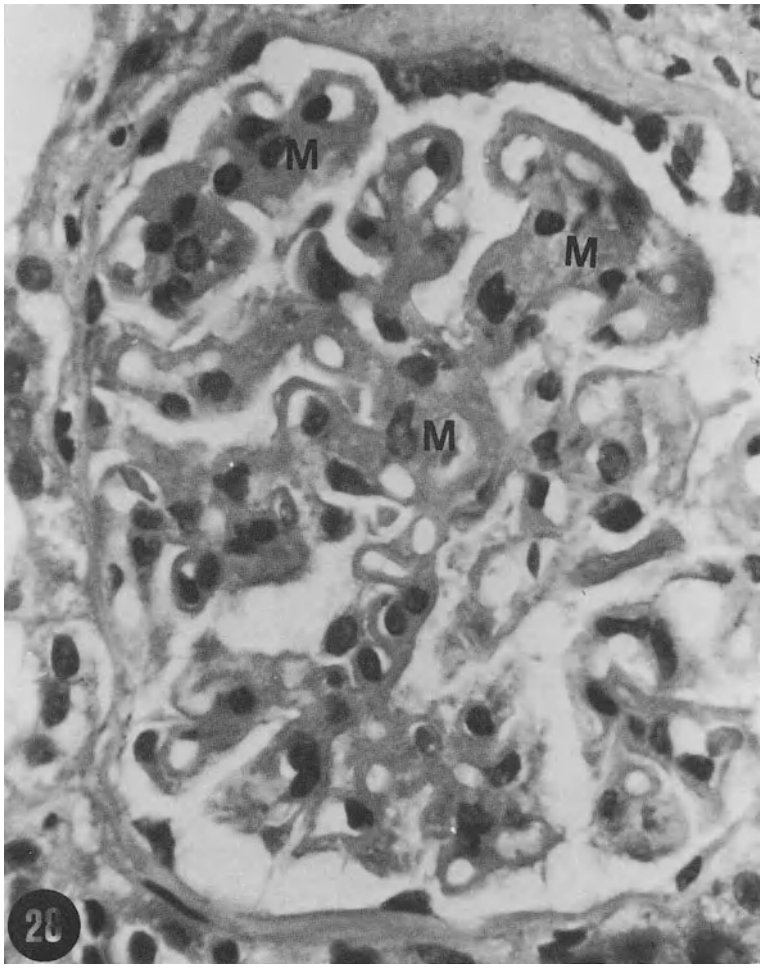

Fig. 6-28. Light micrograph of a glomerulus reveals diffuse and marked thickening of basement membrane of peripheral capillary loops. Moderate increase in mesangial matrix (M) is also noticed. These changes are consistent with stage 3 membranous glomerulonephritis (H & E, ×400).

LM evaluation in the study of membranous GN, including (1) difficult and unreliable staging and (2) difficult differentiation of idiopathic membranous glomerulonephritis from other types of GN-associated GBM changes resembling those in membranous glomerulonephritis, e.g., mesangioproliferative GN or sclerosing GN.

EM study is immensely valuable in the early stage of membranous glomerulonephritis when the GBM changes are not clearly noticeable by LM study. EM study is especially important in view of implementing corticosteroid therapy, which may be recommended on the basis of mild or no GBM change observed by LM alone, and considering the likelihood of minimal lesions or lipoid nephrosis.

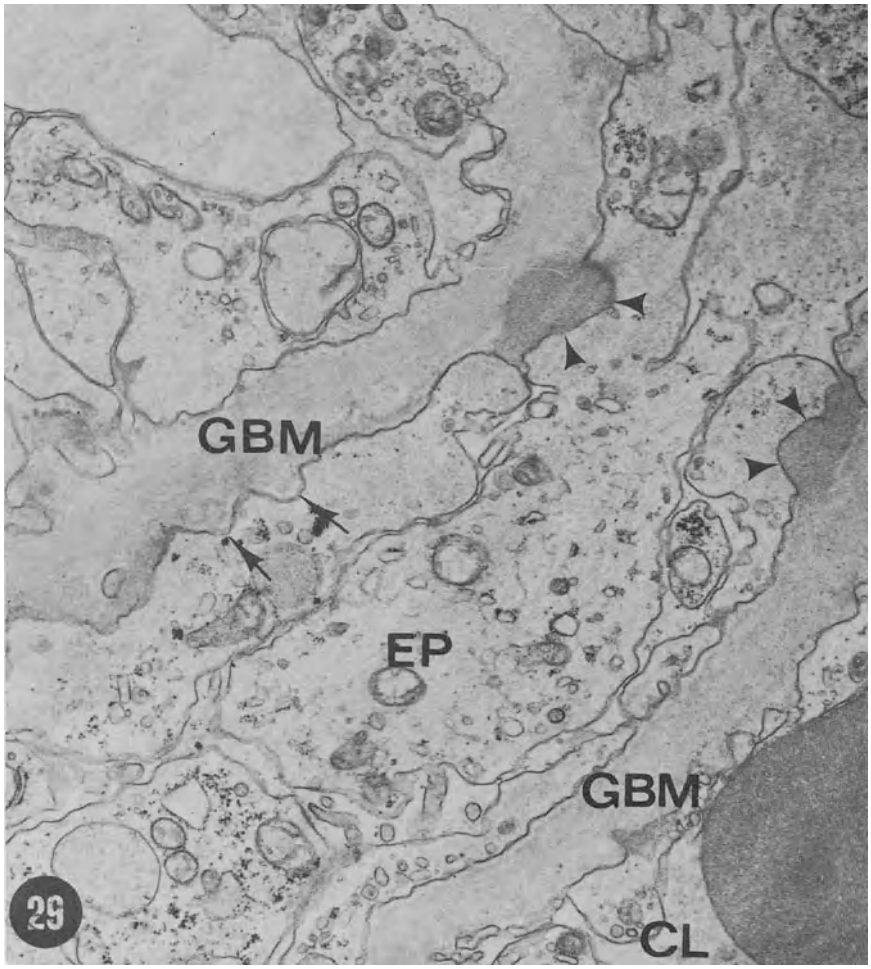

Fig. 6-29. Stage 1 membranous glomerulonephritis. A few extramembranous deposits (arrowheads) and spikes (arrows) are seen. The thickness of glomerular basement membranes (GBM) appears to be within normal limits. Capillary lumen (CL) and epithelial cells (EP) are shown (UA + LC, ×18,000).

Staging of Membranous Glomerulonephritis

The pathological anatomy of membranous GN can be divided into four stages, according to the apparent thickness of GBM and the arbitrary number of spikes and electron-dense deposits found in the GBM:

Stage 1. GBM is of normal thickness or mildly thick; spikes and electron-dense deposits are few and far between; generalized fusion of foot processes and normal amounts of mesangial matrix are observed (Fig. 6-29).

Stage 2. GBM is mildly thick and there are more spikes. The striking feature is numerous electron-dense deposits of similar size and shape, which are present at regular intervals through the GBM (Figs. 6-30 to 6-32).

Stage 3. GBM is markedly thickened and spiky with less numerous electron-dense deposits. The electron density of the deposits is less than that of the deposits in stage 2. The GBM exhibits a sawtooth appearance, and there is a moderate increase in mesangial matrix (Figs. 6-33 and 6-34).

Stage 4. Same as stage 3 except with fewer deposits and more mesangial matrix (Figs. 6-35 and 6-36).

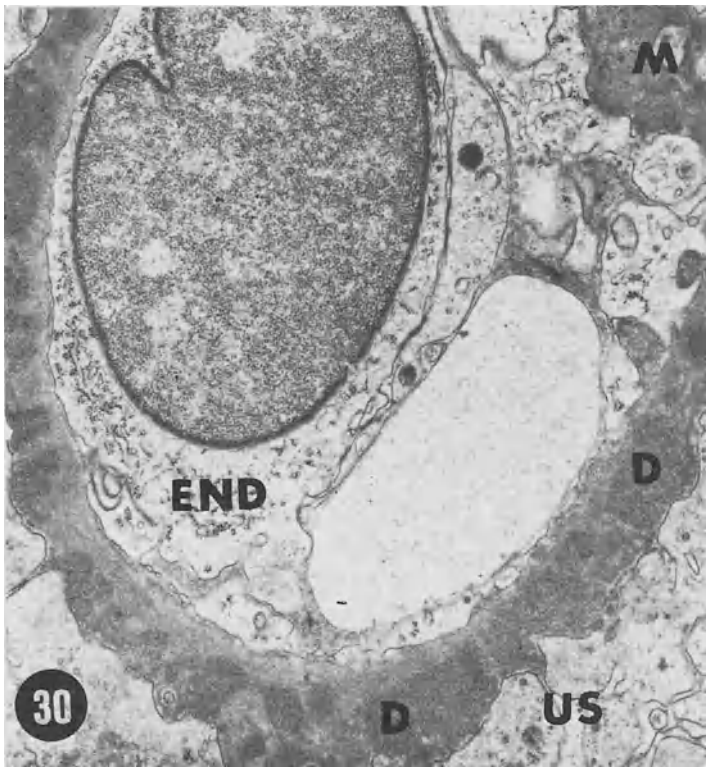

Fig. 6-30. Stage 2 membranous glomerulonephritis is characterized by numerous extramembranous deposits (D) seen at almost equal intervals. Spikes are few and inconspicuous. Swelling of endothelial cell (END) is observed (UA + LC, ×15,000).

In all stages, the tubules appear essentially normal; however, excessive collagen fibers have been noted in the interstitium, especially when membranous glomerulonephritis is associated with renal vein thrombosis (Fig. 6-37). Thickening of the basement membrane and the presence of electron-dense deposits within the basement membranes of the arterioles have been observed in stages 3 and 4 membranous glomerulonephritis (see Fig. 12-19b). The clinical implications of the staging of membranous GN are discussed in Chapter 7.

Causes of Membranous Glomerulonephritis

By and large, membranous glomerulonephritis (idiopathic membranous glomerulonephritis) is not preceded by any identifiable antigenic stimuli. A small number (10–15%) of cases of membranous glomerulonephritis occur in association with a variety of illnesses, for example, SLE, secondary syphilis, congenital syphilis, unilateral or bilateral renal vein thrombosis, and viruses, or the use of drugs (e.g., penicillamine, gold).

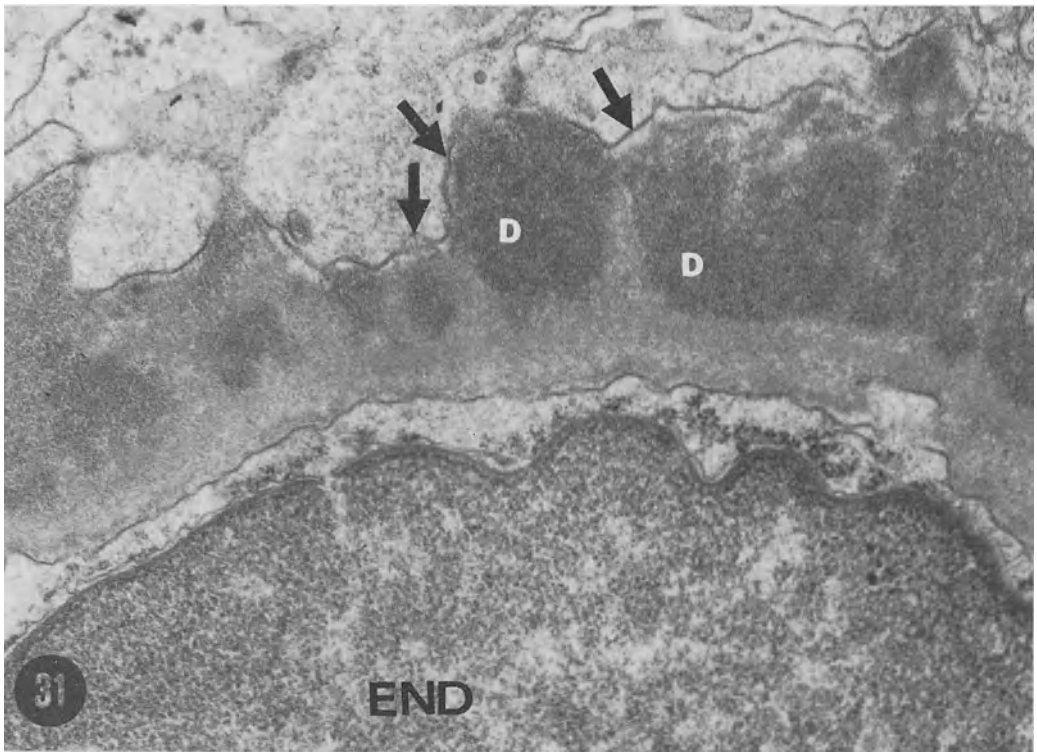

Fig. 6-31. Magnified view of the glomerular capillary shown in Fig. 6-30. The extramembranous deposits (D) stand out prominently. Note the absence of limiting membrane in the deposits. The membranes shown (arrows) are of the foot processes. The nucleus of the endothelial cell (END) can be seen (UA + LC, ×25,000).

With the exception of SLE, the cause and effect relationships between these conditions and membranous glomerulonephritis are largely unconfirmed. There is no serial biopsy study which could document the sequence of development of glomerular basement membrane changes in these conditions. Several isolated patients' reports do not provide any clue to the pathogenetic mechanism, nor can they establish that membranous GN is caused directly by these stimuli. The details of secondary membranous glomerulonephritis are discussed in the chapter on the nephrotic syndrome.

PENICILLAMINE-INDUCED GLOMERULONEPHRITIS

Penicillamine appears to have an adverse influence on the kidney and produces a variety of glomerular diseases ranging from focal glomerulonephritis to membranous glomerulonephritis. An immune complex mechanism has been implicated in the pathogenesis of penicillamine-induced glomerulonephritis, although the following are yet unknown: (1) whether or not the parent penicillamine or a degradation product acts as an antigen, (2) whether the parent drug or the

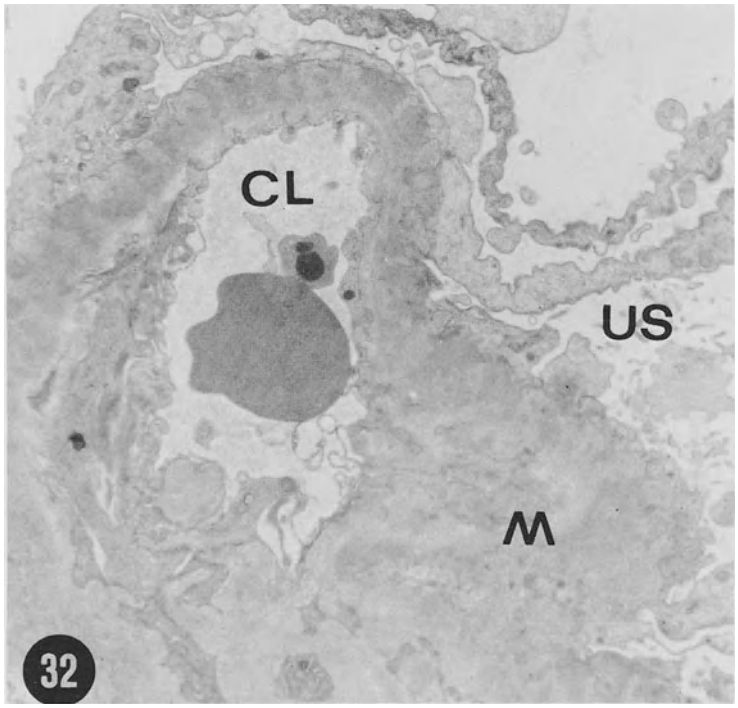

Fig. 6-32. Stage 2 membranous glomerulonephritis in a patient with thrombosis of inferior vena cava and both renal veins. Note the row of electron-dense deposits in the peripheral capillary loop and the slight increase in mesangial matrix (M). Lumen of the capillary loop (CL) and urinary space (US) can be seen (UA + LC, ×12,000).

metabolite stimulates antibody production, and (3) whether the penicillamine alone or other antibiotics, such as penicillin or ampicillin, which may be administered concomitantly for associated infections, potentiate the antigenic effect of penicillamine. An example of penicillamine-induced glomerulonephritis is cited here to illustrate this condition. Although this patient did not reveal typical membranous GM, as did those reported in the literature, she is discussed here because of the close association between penicillamine and membranous GN.

Patient C.S., a 57-year-old white female, was admitted to the University of Oklahoma Hospital on January 2, 1977, with a history of nausea, vomiting, and epistaxis and the laboratory findings of thrombocytopenia and elevated serum urea nitrogen. Twenty-seven months prior to this admission, she was placed on D-penicillamine 250 mg tid for rheumatoid arthritis. Serial urinalysis demonstrated 3+ proteinuria 16 months after initiation of therapy. Two months prior to admission, she noticed bloody urine and felt less efficient

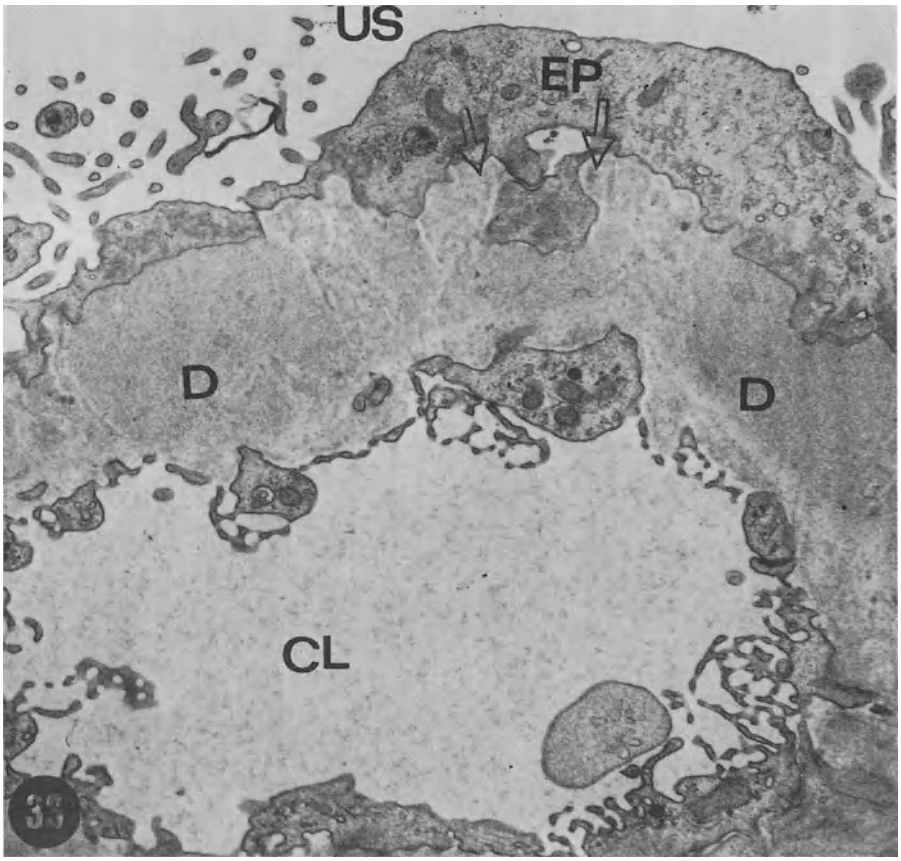

Fig. 6-33. Electron microscopy of the glomerulus shown in Fig. 6-28. The EM confirms the light microscopy assessment of stage 3 membranous glomerulonephritis. The deposits (D) are fewer and not as electron dense as in Figs. 6-30 to 6-32. The ragged glomerular basement membrane reveals conspicuous spikes (arrows). The epithelial cells (EP) and foot processes are fused. The lumina of the capillary loop (CL) and the urinary space (US) are seen (UA + LC, ×19,000).

and confused. Three days prior to admission, recurrent epistaxis compelled her to see a physician. A diagnosis of sinusitis was made and she was treated with oral penicillin. At this visit, a platelet count of 185,000/mm^3 was obtained. Three days later, she felt worse, and her admission to the University of Oklahoma Hospital was warranted by the rapid fall in platelet count (55,000/mm^3) and rise in serum urea nitrogen. She was normotensive at the time of initiation of penicillamine therapy but became hypertensive 8 months later. Treatment was started with reserpine and hydrochlorothiazide, but was later discontinued. At the time of admission she was receiving Catapres (clonidine) and Dyazide (a combination of triamterence and hydrochlorothiazide) in addition to penicillamine and penicillin.

Physical examination revealed numerous ecchymoses over the arms, and across the abdomen and back. The remaining physical examination was essentially normal. Stool was positive for blood. A chest x-ray and an ECG were found to be normal.

Her admission laboratory studies were as follows: Hemoglobin 11.1 g/100 ml, WBC 10,400/mm^3, platelet count 55,000/mm^3; urinalysis: specific gravity 1.015, protein 2+, numerous RBC and WBC, and 0–2 RBC casts/HPF; serum chemistry: urea nitrogen 189 mg/100 ml, creatinine 17.4 mg/100 ml, Na^+ 145 mEq/liter, K^+ 3.7 mEq/liter, Cl^- 102 mEq/

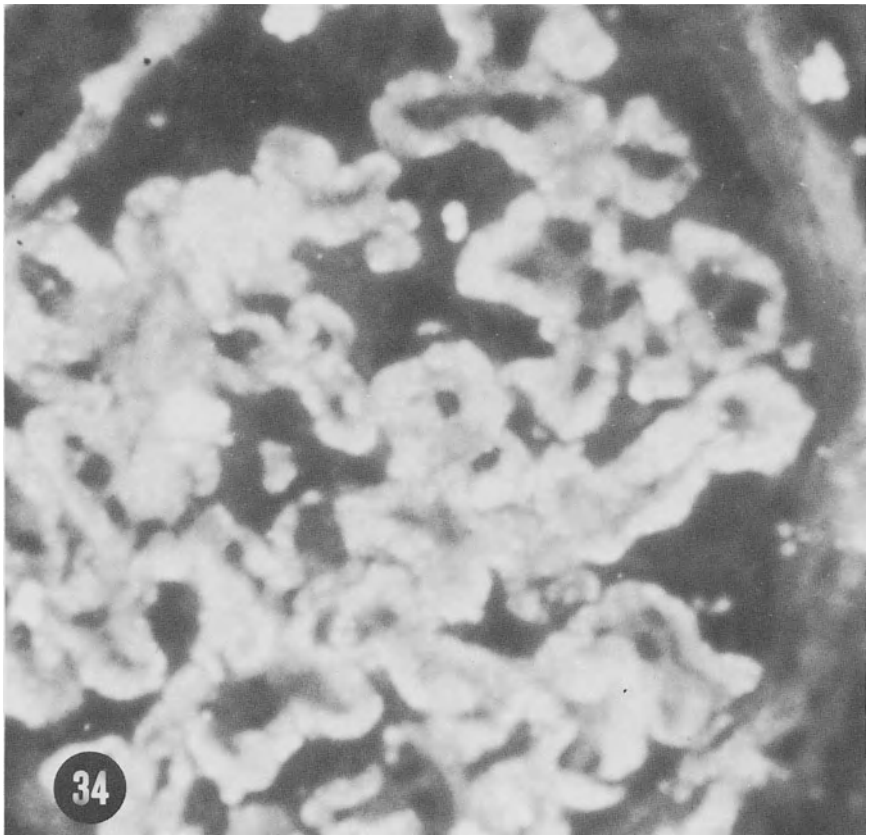

Fig. 6-34. Conspicuous granular fluorescence of IgG in the basement membrane, diffusely throughout the glomerulus. The granular appearance is more discrete in the capillaries in the middle portion than in the upper or lower portions of the glomerulus (IFM, ×1600).

liter, CO_2 14 mEq/liter. Other tests included negative LE cell preparation, VDRL, and rheumatoid factor; positive antinuclear antibody with a titer of 1:160; and double-stranded anti-DNA antibody with a titer of 26.5 units/ml (normal value = 25 units/ml) and low serum C3 (117 mg/100ml).

She was treated initially with peritoneal dialysis and subsequently with hemodialysis. She received ampicillin for suspected urinary tract infection from the fifth through the ninth hospital day. On and after the 16th hospital day, her urine output increased and serum creatinine began to fall. On the 23rd hospital day, a percutaneous renal biopsy was done and the tissue was fixed for LM, EM, and IFM studies. On the 27th hospital day,

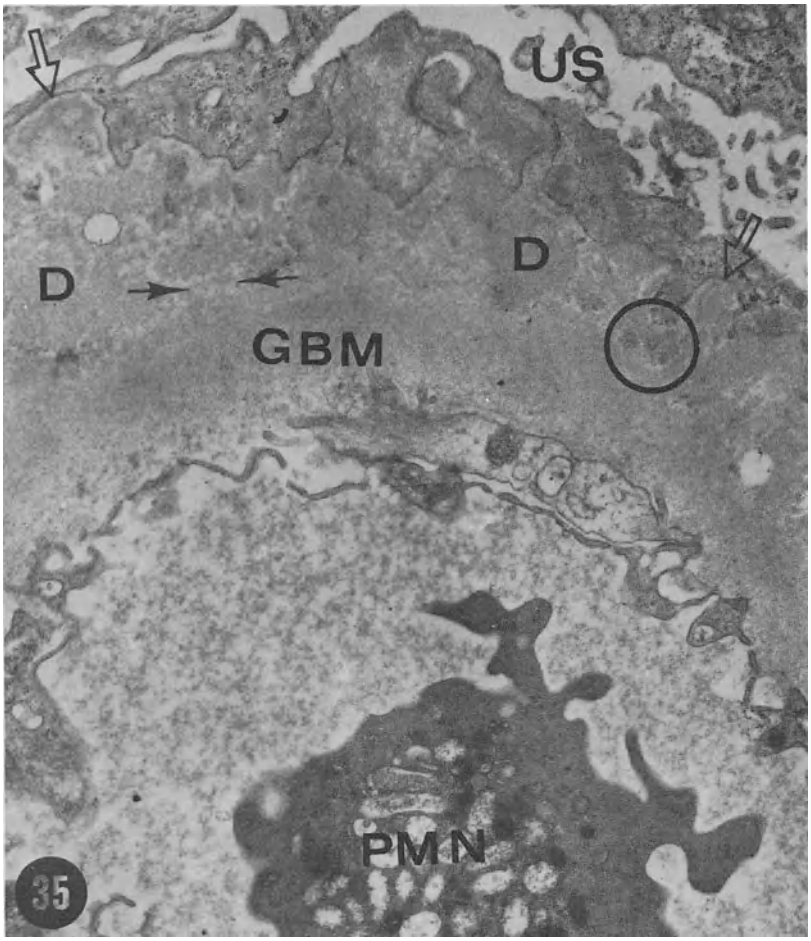

Fig. 6-35. Stage 4 membranous glomerulonephritis. Note the scarcity of deposits (D), even with slight electron density. The basement membrane (GBM) appears to consist of two layers with a clear division (opposing arrows) between the two, a smooth inner layer below the arrows and a ragged outer layer above the arrows. Prominent spikes (open arrows) are shown. Small spherical structures (circle) mimic viral particles (see later for viral particles). Part of a neutrophil leukocyte (PMN) can be seen within the lumen. Epithelial cell and foot processes are fused with the basement membrane. Urinary space (US) is shown (UA + LC, ×19,000).

her ANA titer decreased (1:40) but serum C3 dropped further (100 mg/100 ml). From the 29th hospital day, prednisone 40 mg/day orally was added to her regimen. Bactrim was started on the 33rd hospital day for significant colonies of *Proteus* grown in the urine culture. She continued to have a low-grade fever for which cultures of blood, urine, and sputum were done. By the 39th hospital day, her urinary output increased to 1700 to 1800 ml/day and her serum creatinine decreased to 5.5 mg/100 ml. She developed a spiking fever for which she was placed on gentamicin and ampicillin. She received two doses of ampicillin, 1 g each, intravenously. On the following day, she became anuric and her serum creatinine increased to 7.0 mg/100 ml. A third administration of ampicillin was followed immediately by shaking chills, pruritic macular rash on the feet and arms, and fast deterioration of renal function, which required hemodialysis treatment. Serum C3 remained low (100 mg/100 ml). No more ampicillin was administered. Thereafter, her renal function gradually improved but did not reverse to normal. She was discharged from the hospital 2 months after admission. Follow-up 7 months after her initial visit to the University Hospital revealed a normal physical examination: Urinalysis: 4+ protein, and 4 to 7 WBC, 20 to 35 RBC, 3 to 6 hyaline casts, and 0 to 2 granular casts/HPF; serum urea nitrogen 84 mg/100 ml with normal electrolytes and CO_2; normal serum C3 (135 mg/100ml);

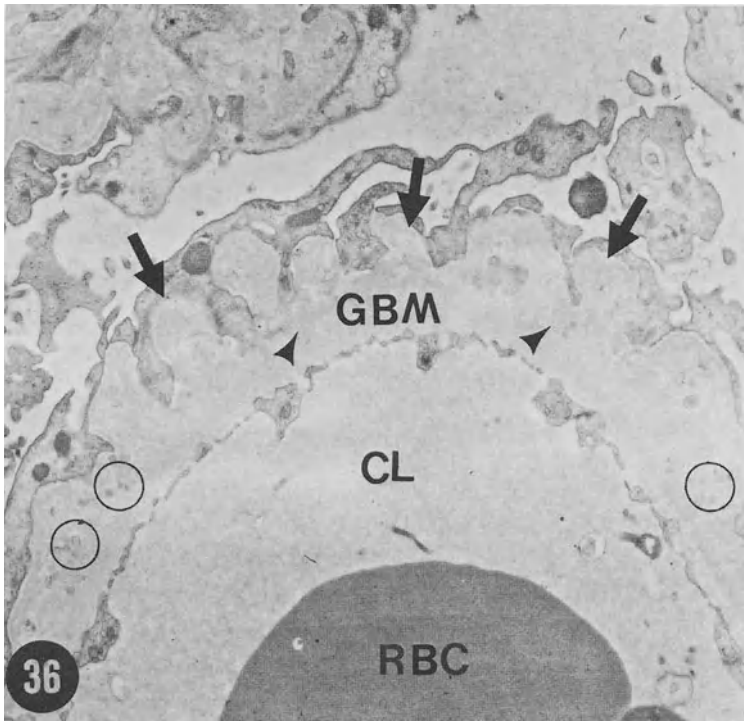

Fig. 6-36. Stage 4 membranous glomerulonephritis from a patient 1 year after the first biopsy, which revealed stage 2 membranous glomerulonephritis (Fig. 6-32). Note conspicuous spikes (arrows) and few or no deposits (arrowheads). These have given a sawtooth appearance to the glomerular basement membrane (GBM). The spherical structures (circles) mimic viral particles. Red blood cells (RBC) are seen within the lumen of the capillary (CL) (UA + LC, ×12,000).

and negative ANA titer. She is seen in the renal clinic periodically and receives conservative management for chronic uremia.

Renal tissue was studied by LM, EM, and IFM. LM study showed prominent crescents in one-half to two-thirds of the total number of glomeruli. The crescents were mainly cellular. A few glomeruli showed segmental cellular proliferation with an appearance suggestive of necrosis (Fig. 6-38). A few tubules were observed, some of them containing casts impregnated with neutrophil leukocytes and RBC. The interstitium was prominent because of fibrosis and infiltration (mainly by lymphocytes, and also some plasma cells and occasional neutrophils, but no eosinophils). Arterial vessels were unaffected. All the glomeruli in the methylene blue–azure II-stained thick sections revealed segmental necrotic and proliferative lesions involving one-third to three-fourths of the glomeruli. Two different glomeruli were studied by EM. In both glomeruli, most of the capillaries were

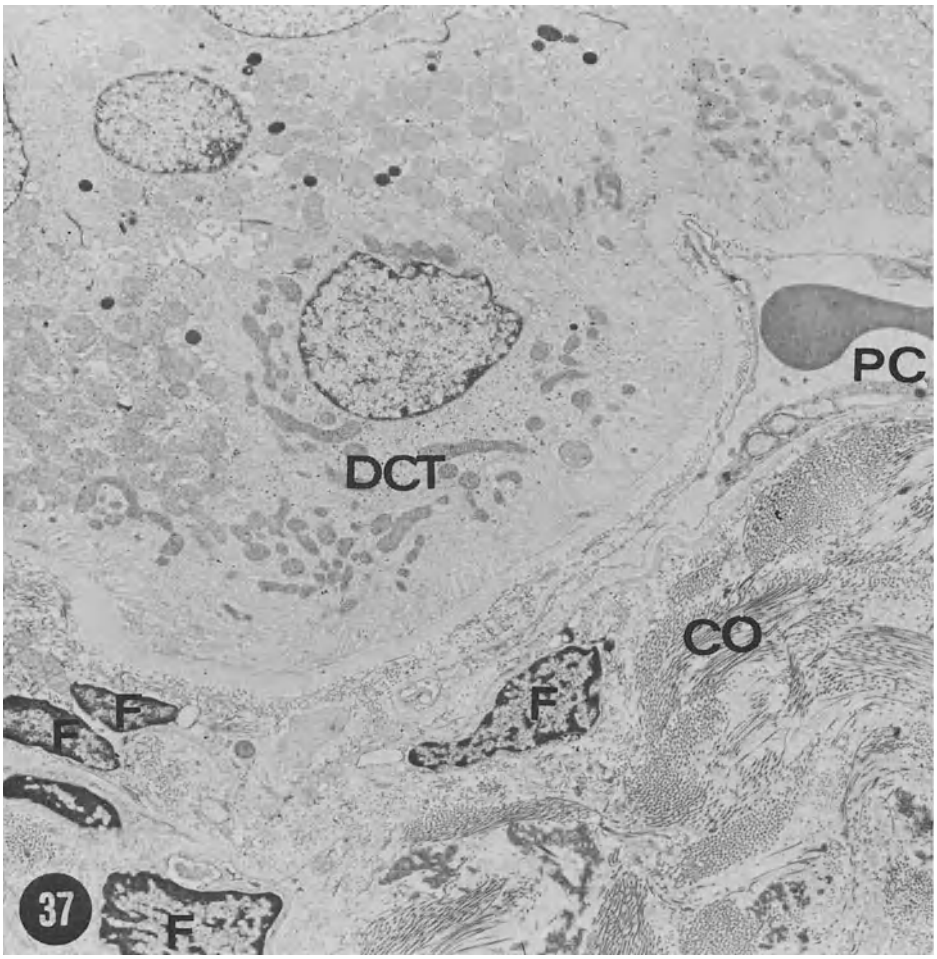

Fig. 6-37. Normal distal convoluted tubule (DCT) and a peritubular capillary (PC). Note the excessive number of fibroblasts (F) and excessive amounts of collagen fibers (CO) in the interstitium (UA + LC, ×6000).

abnormal with a few having normal appearance. The abnormalities were characterized by irregular thickening of the endothelial aspect of the GBM in some capillaries, spikes of the GBM in other capillaries, and electron-dense deposits of variable size within the GBM and in extramembranous and subendothelial locations of the GBM. The ultrastructural features of all stages of membranous GN have been observed in more than half of the total number of glomerular capillaries (Figs. 6-39 and 6-40). Collapse and fragmentation of GBM were noted. Marked proliferation of Bowman's epithelium and adhesion of Bowman's epithelial cells to the visceral epithelial cells (crescent formation) were observed. Proliferation of endothelial cells was marked in some of the capillaries. Most of

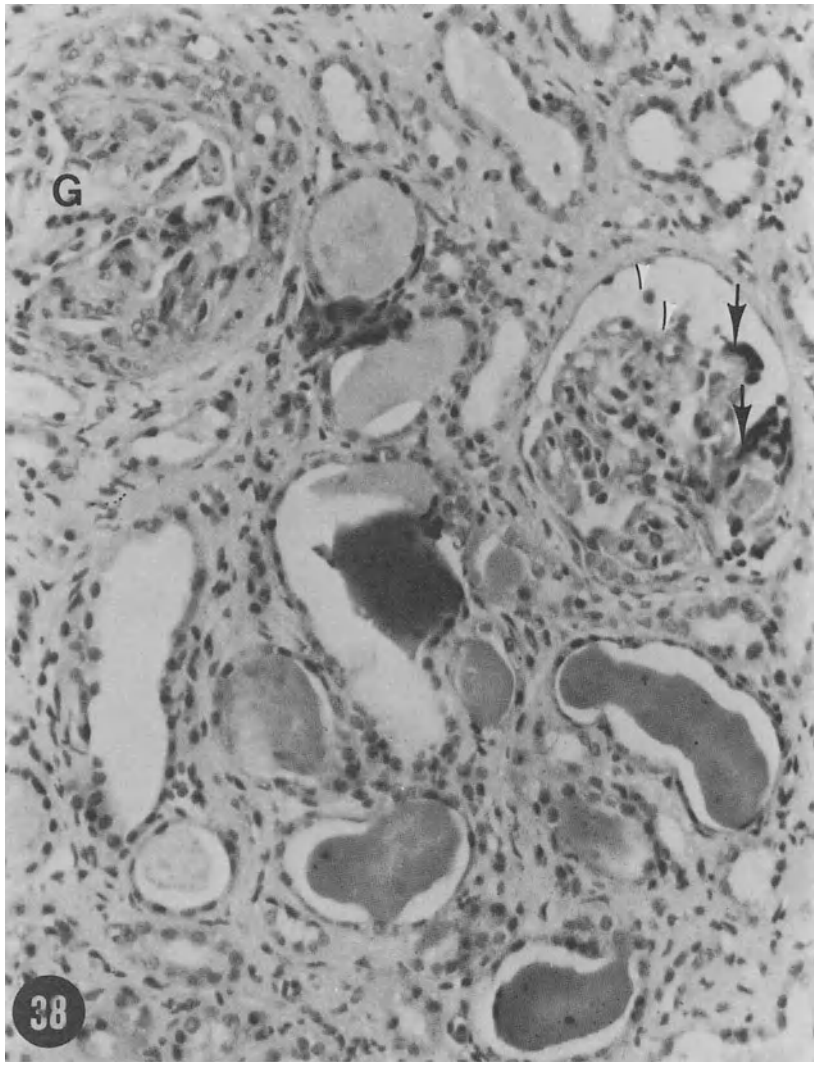

Fig. 6-38. This micrograph shows crescent and necrosis of the capillaries in glomerulus (G), dark nuclei which appear to be hematoxylin bodies (dark arrows), and segmental necrosis (arrowheads) in another glomerulus. Most of the tubules are dilated and filled with casts (H & E, ×120).

the tubules showed atrophic changes with thickening of the tubular BM (i.e., basement membrane of tubules). The interstitium revealed edema and infiltration by fibroblasts, collagen fibers, plasma cells, and occasional neutrophil leukocytes.

IFM study revealed granular fluorescence for IgG, IgM, and C3 in the glomerular basement membrane. However, the fluorescence for IgG was brighter than that for IgM or C3. Bright fluorescence for fibrinogen was found in the crescents, but no fluorescence for IgA or IgE was observed.

Comment

This report describes immune complex glomerulonephritis and irreversible renal failure, which apparently developed following treatment with penicillamine for rheumatoid arthritis. The LM histology with prominent crescent formation

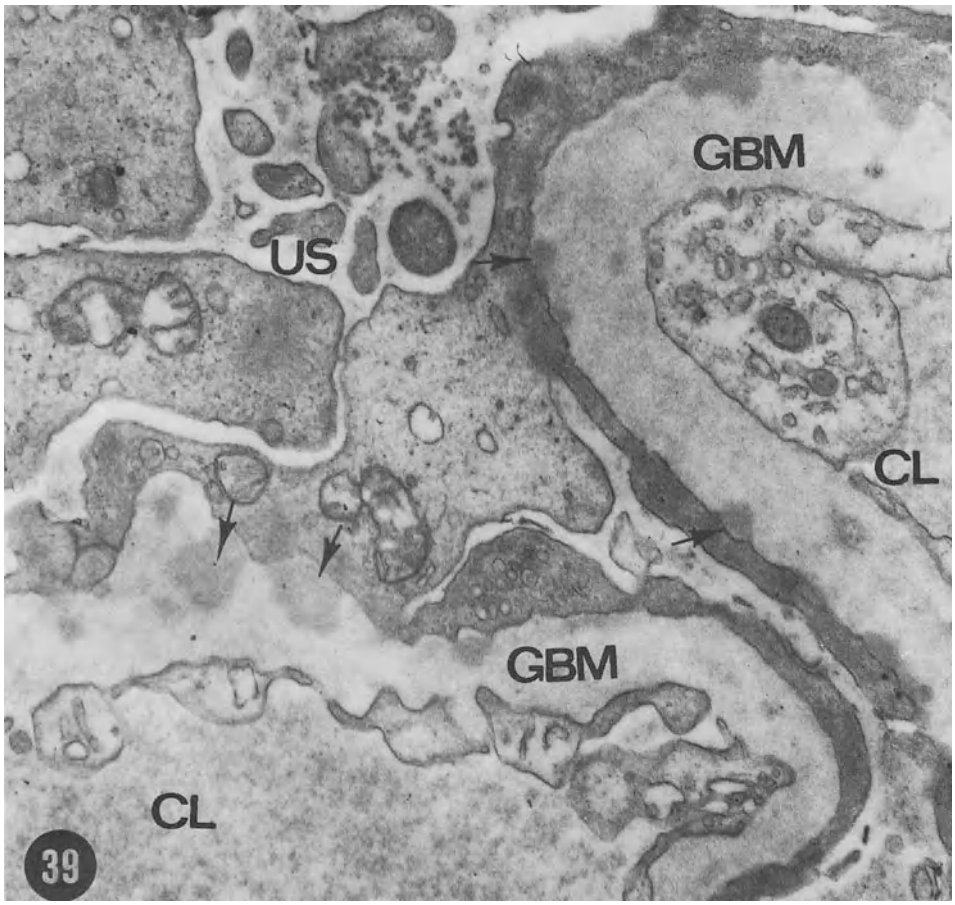

Fig. 6-39. Glomerular capillaries reveal normal to mildly spiky basement membrane (GBM) and a few small extramembranous deposits (arrows). These changes are consistent with stage 1 membranous glomerulonephritis. Lumen of the capillary (CL) and urinary space (US) are shown (UA + LC, ×19,000).

involving 50 to 75% of the glomeruli was consistent with rapidly progressive glomerulonephritis (RPGN). The tubulointerstitial changes were supportive of this diagnosis. The EM study revealed a picture of mixed proliferative and membranous GN or membranous transformation of proliferative GN. The findings of excessive amounts of intramembranous, extramembranous, and subendothelial deep electron-dense deposits, along with endocapillary proliferative and nuclear degenerative changes, were compatible with lupus nephritis. This diagnosis, which was obtained from EM study, was supported by IFM findings, as well as by elevated titers of double-stranded anti-DNA antibody and antinuclear antibody but negative rheumatoid factor.

The intriguing feature was the questionable influence of penicillin, ampicillin, and/or gentamicin on the clinicopathological syndrome in this patient. Acute interstitial nephritis and oliguria have been reported after penicillin. Immune

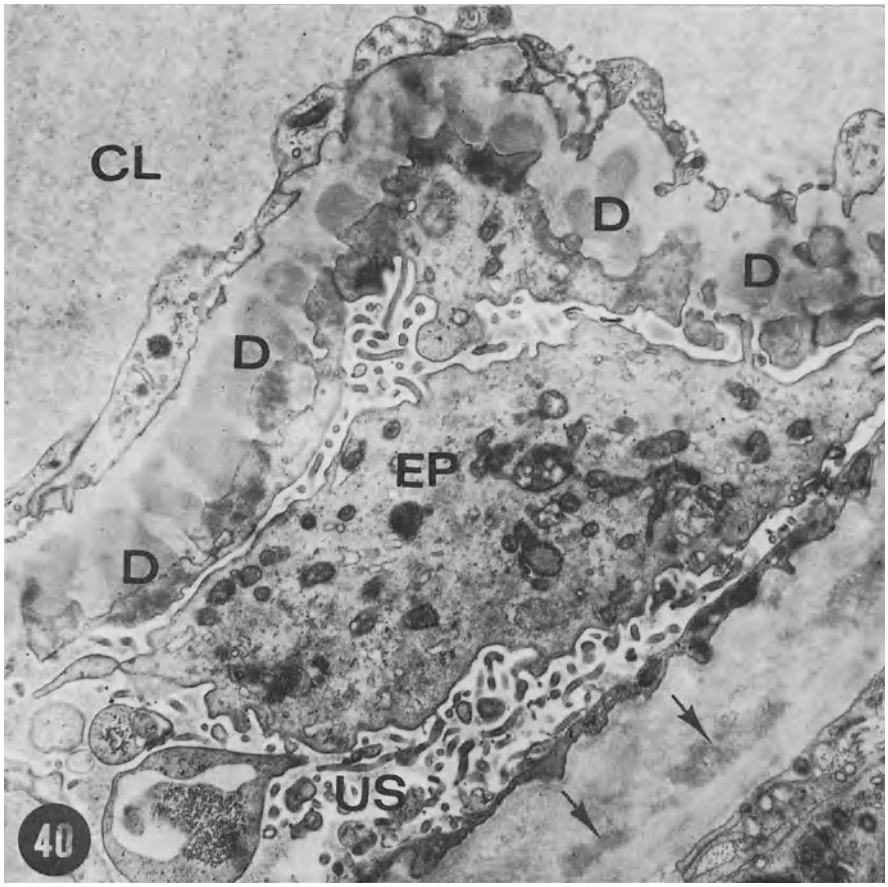

Fig. 6-40. In this capillary loop, numerous extramembranous deposits (D) suggest stage 2 membranous glomerulonephritis. Note deposits (arrows) in Bowman's membrane. Lumen of the capillary (CL), epithelial cell (EP), and urinary space (US) are shown (UA + LC, ×16,000).

complex glomerulonephritis similar to that described herein, along with acute interstitial nephritis, after penicillin and kanamycin has been reported. Penicillamine differs chemically from penicillin and ampicillin but cross-sensitivity is known to occur among these compounds. Worsening of symptoms and appearance of thrombocytopenia after administration of penicillin, deterioration of renal function following ampicillin, and the improvement of the renal function after the withdrawal of ampicillin suggest antigenic potentiality of these drugs in this patient. It is then conjectured that penicillin or ampicillin, or both, through cross-reactivity with penicillamine, may stimulate excessive antibody production and immune complex glomerulonephritis.

MESANGIOPROLIFERATIVE GLOMERULONEPHRITIS

Mesangioproliferative glomerulonephritis (MPGN) is a type of immune complex glomerulonephritis in which glomerular mesangium bears the brunt of the disease process. There are numerous synonyms which must be kept in mind in order to avoid error and confusion: (1) membranoproliferative glomerulonephritis, (2) mixed proliferative and membranous glomerulonephritis, (3) mesangiocapillary glomerulonephritis, (4) hypocomplementemic persistent glomerulonephritis, (5) dense deposit disease, (6) laminar glomerulonephritis, and (7) lobular glomerulonephritis (Allen, 1966).

The terms membranoproliferative and mixed proliferative and membranous glomerulonephritis are inappropriate because both imply basement membrane changes suggestive of membranous glomerulonephritis, which is not the usual finding in this type of glomerulonephritis. Hypocomplementemic glomerulonephritis is the clinical denominator for the pathological process. Dense deposit disease is the ultrastructural appearance of the glomerular and tubular basement membranes in glomerulonephritis generally associated with hypocomplementemia. Laminar glomerulonephritis is a mere morphological description of the capillary basement membrane change.

Therefore, it appears that lobular glomerulonephritis is the most appropriate morphological term to denote mesangioproliferative glomerulonephritis. The term lobular glomerulonephritis was applied first in 1951 to the sclerotic glomerular lesion, described variously by Bell as "chronic azotemic," "hydropic," "latent," or simply "chronic glomerulonephritis." Allen (1966) elaborated this pathological process in his textbook on the kidney, and his vivid description of this pathological process still holds. In a recent communication (1976), he stated that lobular glomerulonephritis consisted not of a "simplification" of glomerular structure or of "mesangial" or "axial" proliferation but of a complex combination of alterations in the networks of centrilobularly located walls of the glomerular capillaries along with their attendant, principally endothelial, cells. The changes in the walls ranged from minimal thickening to a marked fibrinoid degeneration with progressive coalescence and eventual sclerotic obliteration of the lumens of the centrilobular capillaries.

I agree with Dr. Allen and would like to suggest further that lobular glomerulonephritis is not merely a term to describe glomerular structure but a complex pathological process which involves the centrilobular portions of the glomerular capillaries mainly and leads to this morphological entity. Our experience suggests that lobular glomerulonephritis is more closely related to hypocomplementemia, which is dealt with in detail later.

The lobular response of the glomerulus to streptococcal tonsilitis in an adult with a preceding membranous glomerulonephritis and nephrotic syndrome was documented by Allen in 1955. The association of the lobular glomerulonephritis with streptococcal infections has been described by others. This association is common, but is by no means specific. The lobulation pattern of glomeruli similar to that seen in lobular glomerulonephritis has been documented by several observers in patients who fail to heal after an attack of acute poststreptococcal glomerulonephritis. The published observations do not support streptococcal infection as an important etiological factor in the pathogenesis of lobular glomerulonephritis; therefore, most individuals like to describe this as idiopathic mesangioproliferative GN. The argument in favor of the association of this pathological process with a low level of third component of serum complement (C3) is still valid. Thus, hypocomplementemia is considered by many investigators as a distinct factor in the pathogenetic mechanism, but even so it is often denoted as hypocomplementemic glomerulonephritis. Jones (1977) believes that mesangioproliferative GN may occur in association with a variety of infectious diseases, including bacterial endocarditis, shunt infection, malaria, and so on; neoplastic conditions such as leukemia and lymphoma; sickle cell anemia; and drug abuse such as heroin addiction.

All types of mesangioproliferative GN assume a more or less lobular pattern. This is most convincingly observed in thick (survey) sections prepared for EM study. Allen (1955) has stated that this morphology was frequently misinterpreted as nodules of diabetic glomerulosclerosis. The differential histological features are indicated in the illustrations in this chapter and the chapter on the nephrotic syndrome.

There exist numerous controversies concerning the pathological types and the morphological descriptions of MPGN. The author has attempted to bridge the different views for a more constructive proposition directed toward clinical practice.

LM Study

Using LM, three types of mesangioproliferative GN can be recognized:

1. *Lobular glomerulonephritis.* This is characterized by marked accentuation of the lobules and sometimes by complete separation of the lobules of the glomeruli. The lobules show marked hypercellularity, moderate to marked increase in mesangial matrix, and obliteration of many capillarly lumina. Mesangial cells and matrix encroach on the capillaries and cause more difficulty in evaluating

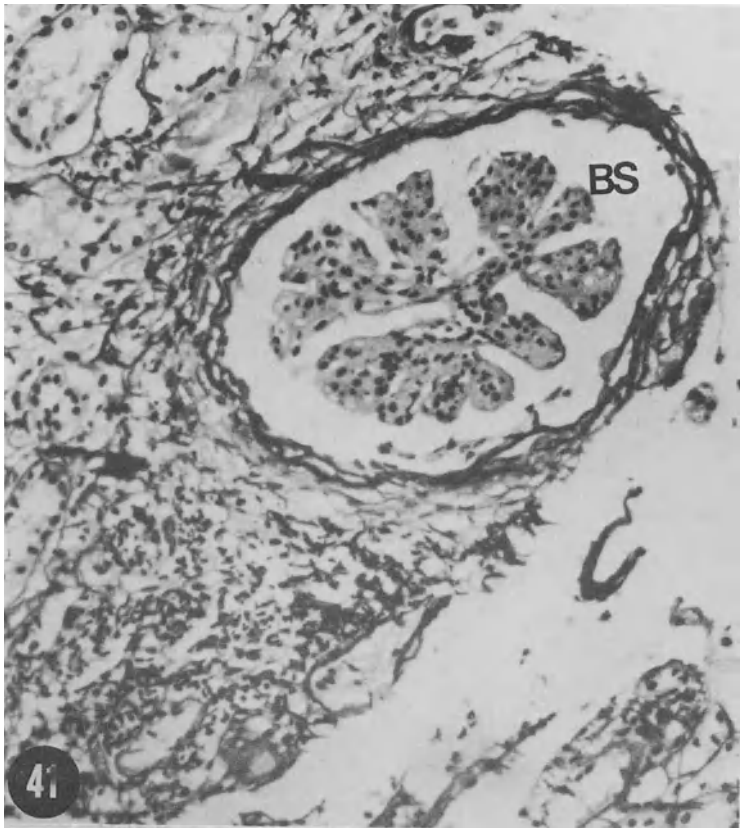

Fig. 6-41. Marked lobulation of the glomerular tufts associated with hypercellularity and excessive matrix of the mesangium. Large Bowman's space (BS) is apparently due to contraction of the glomerulus. An area of cellular infiltration is seen in the interstitium (PAS, ×80).

the peripheral capillary loops. The Bowman's space is enlarged due to contraction of the glomerular tufts. Seldom, if ever, is a crescent seen in this type of glomerulonephritis (Fig. 6-41).*

2. *Dense deposit disease.* This is identified by the homogeneous appearance of all glomerular capillaries, which are highly eosinophilic and PAS positive. The tubular basement membranes are also markedly and irregularly thickened, homogeneous, highly eosinophilic, and PAS positive. Mesangium is not so prominent because of the mild or moderate increase in mesangial matrix.

3. *Mesangiomembranous type.* This type reveals changes characteristic of membranous glomerulonephritis in the peripheral portions of some of the capillary loops. In addition, the GBM reveals widening and splitting that have variously been described as giving it a "railroad" or "train track" appearance.

* Figures 6-41 through 6-51 are micrographs from patients with mesangioproliferative glomerulonephritis.

Prominent mesangium with marked increase in mesangial matrix and cellularity is an obvious finding.

EM study can separate mesangioproliferative GN into two definite types and one less precise type, a classification that is consistent with that of most other observers: (1) type 1, with predominant mesangial change and mesangialization of peripheral capillary loops; (2) type 2, with transformation of glomerular and tubular basement membranes into electron-dense masses; and (3) a less specific type, with a combination of mesangial and membranous changes.

The characteristic features of type 1 are as follows:

1. Parallel basement membranes with interposition of cell(s) resembling mesangial cells. The appearance simulates mesangium and has been aptly described by Jones (1977) as mesangialization of peripheral capillary loops. It has been described by various sources as a "railroad" or a "train track" after conventional LM study. This change is most conspicuous and is seen in almost all capillaries in the advanced stage of the disease process (Figs. 6-42 and 6-43).

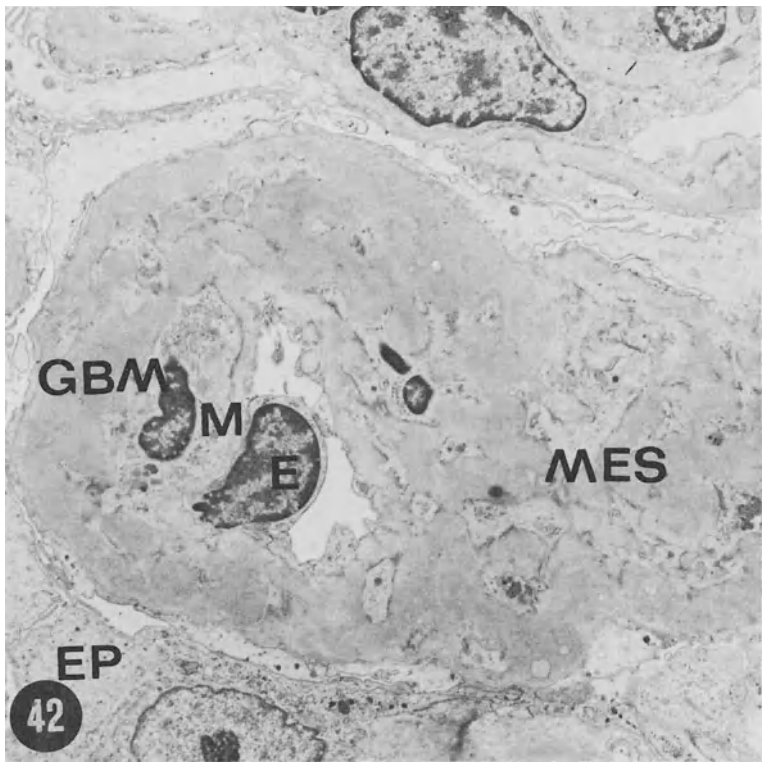

Fig. 6-42. Type 1 mesangioproliferative glomerulonephritis. The micrograph demonstrates mesangialization of peripheral capillary loops. Note endothelial cell (E) and a similar cell called mesangial cell (M) interposed between E and glomerular basement membrane (GBM). GBM shows split or double contour appearance. There is excessive matrix in the mesangium (MES); epithelial cell (EP) is shown (UA + LC, ×3200).

2. Marked increases in mesangial matrix (Fig. 6-42).
3. Large amounts of subendothelial electron-dense deposits (Fig. 6-44).
4. Intramembranous and subepithelial electron-dense deposits, including typical "humps" (Fig. 6-6); however, the latter is a rare finding.
5. Thickening of the basement membranes of the tubules and deep electron-dense deposits (Figs. 6-45 and 6-46).

The characteristic features of type 2, or dense deposit disease, include the following:

1. Excessive amounts of deep electron-dense deposits are seen throughout the entire extent of the glomerular capillaries. The capillaries appear to be expanded in places by massive amounts of electron-dense deposits spread uniformly throughout the basement membranes (Figs. 6-47 to 6-50).
2. Excessive mesangial matrix (Fig. 6-49).
3. Discrete nodular electron-dense deposits within the mesangium (Fig. 6-49).
4. Conspicuous absence of subendothelial or subepithelial deposits.

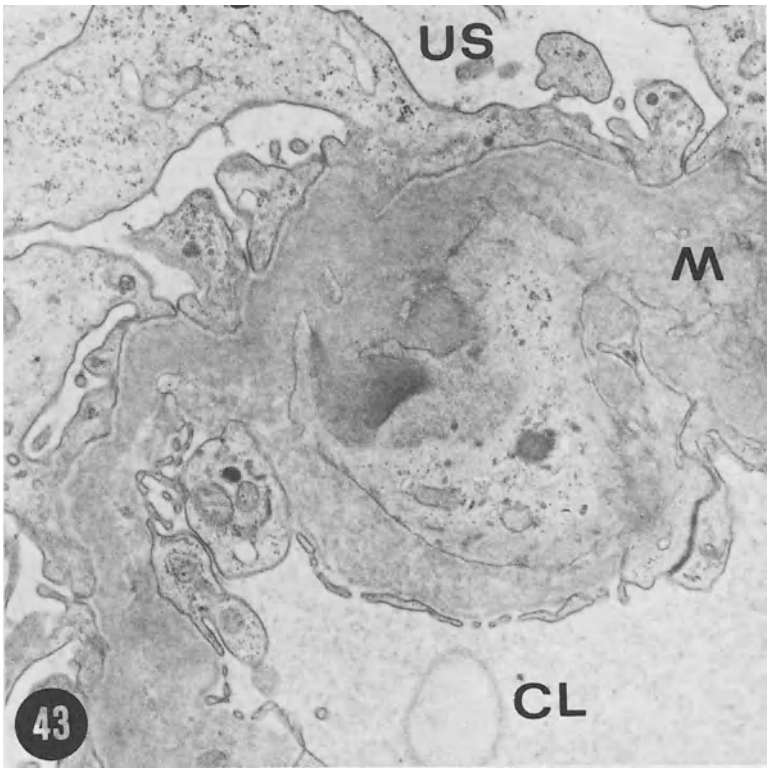

Fig. 6-43. Magnified view of mesangialized (M) peripheral capillary loop. Lumen of the capillary loop (CL) and urinary space (US) are shown (UA + LC, ×12,000).

5. Bowman's membrane and tubular basement membranes reveal appearances similar to those in the glomeruli (Fig. 6-50).

The features of type 3, or nonspecific type, are as follows:

1. One glomerulus may show GBM changes resembling those in membranous GN, whereas another glomerulus may show electron-dense GBM.
2. Conspicuous increase in mesangial matrix.
3. Absence of mesangial electron-dense deposits.

In differentiating between the various types of MPGN, most investigators agree that serum C3 concentration is nearly always very low in type 2 MPGN or dense deposit disease. In type 1 MPGN the serum complement may or may not be depressed, and in type 3 serum complement is almost always normal.

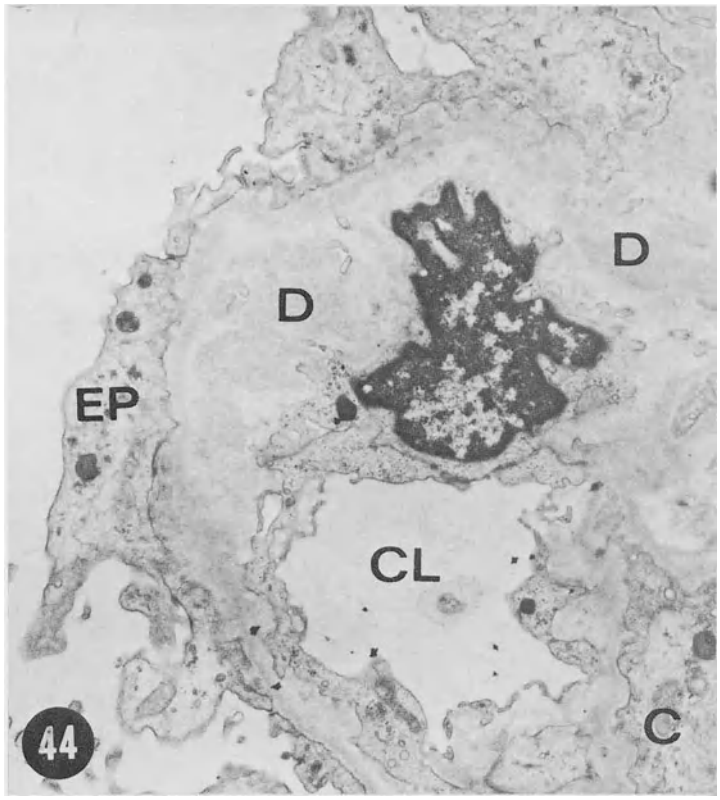

Fig. 6-44. Subendothelial deposits (D) and mesangialized capillary loops (C). Fusion of epithelial cell and foot processes (EP) and lumen of the capillary (CL) are shown (UA + LC, ×8200).

It should be stated that, from the clinical standpoint, all three types of MPGN manifest proteinuria, nephrotic syndrome, hematuria, and renal failure. The clinical and laboratory information from nine patients with MPGN studied by the author and his colleagues is shown in Table 6-11. The results are clinically indistinguishable except for the serum C3 levels. Persistently low serum C3 should alert the clinician to the possibility of dense deposit disease, for a rough correlation seems to exist between the severity of serum C3 reduction and the extent of dense deposits. One patient (L.C.) has very low serum C3 (Table 6-11) and reveals most remarkable and generalized dense deposits in the kidney (Figs. 6-47 to 6-50). According to the opinion of other investigators, as well as in my own experience, it is reasonable to state that the lower the serum C3, greater the amount of deposits found in the glomeruli and tubules. Although almost everyone believes that the course of the disease is relatively more rapid in type 2 MPGN than in either type 1 or type 3, this statement does not always hold true. Thus,

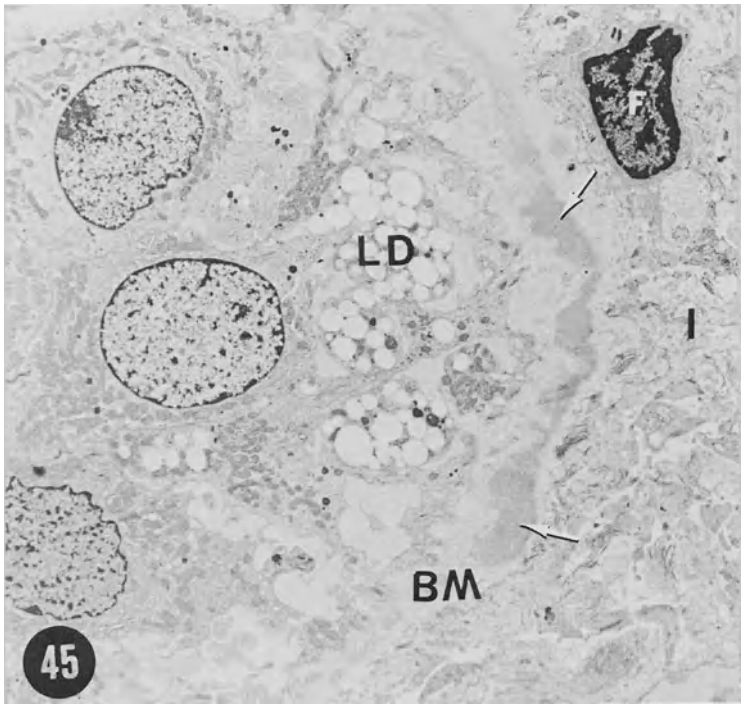

Fig. 6-45. An atrophic distal convoluted tubule reveals electron-dense deposits (arrows) in the basement membrane (BM) and lipid droplets (LD) within the tubular cell. The interstitium (I) is infiltrated with collagen fibers and fibroblasts (F). From a patient with type 1 mesangioproliferative glomerulonephritis. The glomerular EM is shown in Figs. 6-42 to 6-44 (UA + LC, ×3200).

Table 6-11
Clinical and Laboratory Information for Different Types of Mesangioproliferative Glomerulonephritis[a]

Name, age, race, and sex	Initial			Follow-up			
	Proteinuria	SUN/Cr	Serum C3 (mg/100 ml)	EM diagnosis	Duration (mo)	SUN/Cr	Serum C3 (mg/100 ml)
M.A., 17 WF[b]	2+ (UA)	84/5.3	100–105	Type 2 MPGN (dense deposit disease)	60	15/1.0	140
L.C., 11 WF	4+ (UA)	68/1.7	52–58	Type 2 MPGN (dense deposit disease)	46	20/1.5	94
A.C., 49 SAM	10.8 g/24 hr	14/1.2	88	Type 2 MPGN (dense deposit disease)	23	52/3.5	125
D.H., 24 WF[c]	NA	108/5.0	35	Not done	3	20/1.0	120
R.B., 53 WM	6.4 g/24 hr	25/3.2	160	Type 1 MPGN	60	41/5.3	165
L.U., 65 WM	5 g/24 hr	26/1.1	110	Type 1 MPGN	62	33/NA	125
J.B., 19 WF	3.5 g/24 hr	24/2.2	60	Type 1 MPGN	65	170/9.0	54
A.P., 41 WM	9 g/24 hr	26/1.4	190	Type 3 MPGN	4	NA	NA
E.O., 15 WM	1.86 g/24 hr	16/NA	40	Not done	12	16/NA	34

[a] Abbreviations: Cr, serum creatinine (mg/100 ml); MPGN, mesangioproliferative glomerulonephritis; NA, not available; SAM, Spanish-American male; SUN, serum urea nitrogen (mg/100 ml); UA, urinalysis; WF, white female; WM, white male.
[b] Patient M.A. had a kidney transplant in 1973; follow-up was done 60 months after the operation.
[c] Patient D.H. had a kidney transplant in 1973; follow-up was done 3 months after the operation.

in our study, one patient with type 2 MPGN (L.C.) showed improvement of renal function after 46 months, which contrasted with the deterioration of renal function in 65 months in a patient with type 1 MPGN (J.B.).

Despite the distinct correlation between dense deposits in the kidney and serum C3 level, the cause and effect relationship between the two is poorly understood. The frequent absence of immunoglobulins in these kidneys has led to the proposal of an alternative mechanism for the utilization of the complement components, especially C3. This hypothesis, which has been widely accepted, states that excessive breakdown of C3 is caused by a properdin–zymosan complex. This also explains low serum properdin levels in this pathological process; in addition, low serum properdin levels may be accounted for, at least in part, by the presence of properdin deposits in the glomeruli. Deposits of C1 and C4 as well as C3 have been found in the glomeruli. There is very little evidence concerning the involvement of C2 in this pathological process, but whatever the relationship, serum C3 remains persistently low. However, it should be mentioned that serum C3 level often varies but seldom, if ever, normalizes.

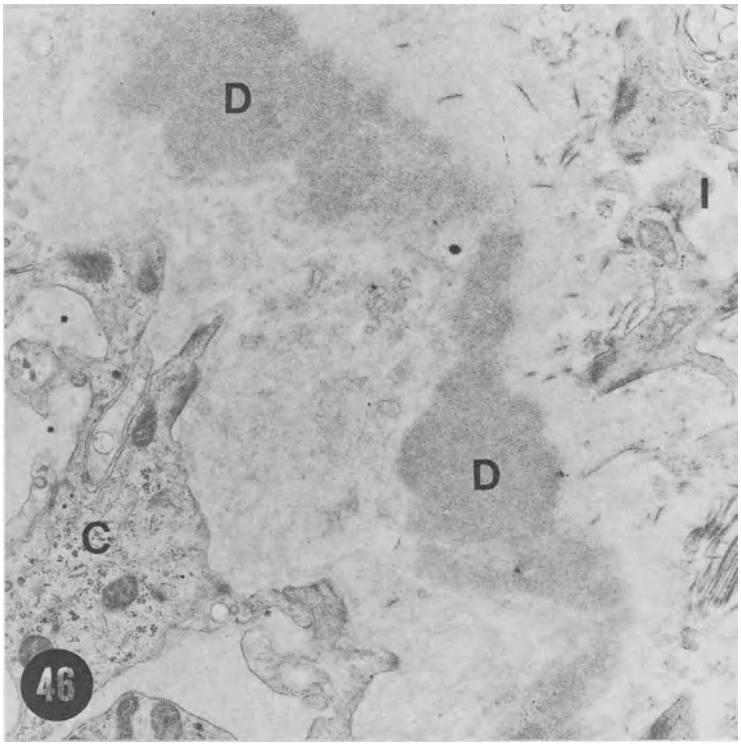

Fig. 6-46. Magnified view of the tubule from the same patient as in Figs. 6-42 to 6-44. The electron-dense deposits (D) in the basement membrane are clearly discerned. The atrophic tubular cell (C) and the fibrotic interstitium (I) are shown (UA + LC, ×15,000).

The presence of abundant complement (C3) in the glomeruli has led many to believe that complement is involved in the pathogenesis of the glomerular lesions. This notion is supported by the observations of a recurrence of mesangioproliferative glomerulonephritis in transplanted kidneys. In one series, renal histology was evaluated in 12 of 16 patients with renal transplants. Among the seven patients showing membranoproliferative GN by LM study, four were found to have hypocomplementemia. One patient of this latter group demonstrated marked hypocomplementemia and kidney with intramembranous deposits typical of dense deposit disease. Another patient who had persistent hypocomplementemia showed progression from predominantly mesangial glomerular changes to

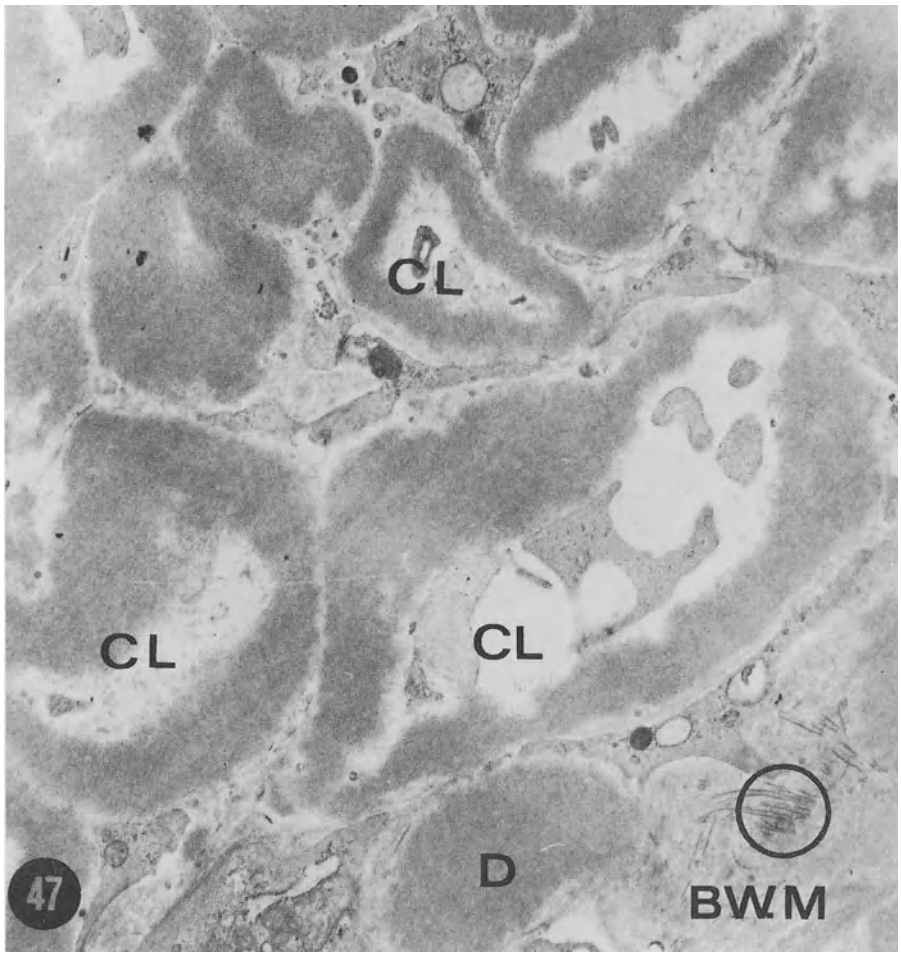

Fig. 6-47. All the capillaries have been transformed into electron-dense masses with loss of epithelial and endothelial cells. A large deposit (D) and collagen fibers (circle) are seen in the Bowman's membrane (BWM). Lumen of the capillaries (CL) is seen (UA + LC, ×8000).

both capillary wall and mesangial abnormalities. The serum complement levels did not improve in hypocomplementemic patients after transplantation.

Since serum C3 has normalized following renal transplants in two patients in our series (Table 6-11, Fig. 6-51), it may be argued that low serum C3 is caused by deposits in the kidney. Even so, it does not explain why C3 is deposited in the kidney in the first place. Our observations at least suggest that normalization of serum C3 levels may serve as a useful index of the freedom from recurrence of MPGN in the grafted kidney.

FOCAL GLOMERULAR SCLEROSIS

SYNONYMS. Focal glomerulonephritis, sclerosing glomerulonephritis, focal sclerosing glomerulopathy with segmental hyalinosis, focal segmental glomerulosclerosis.

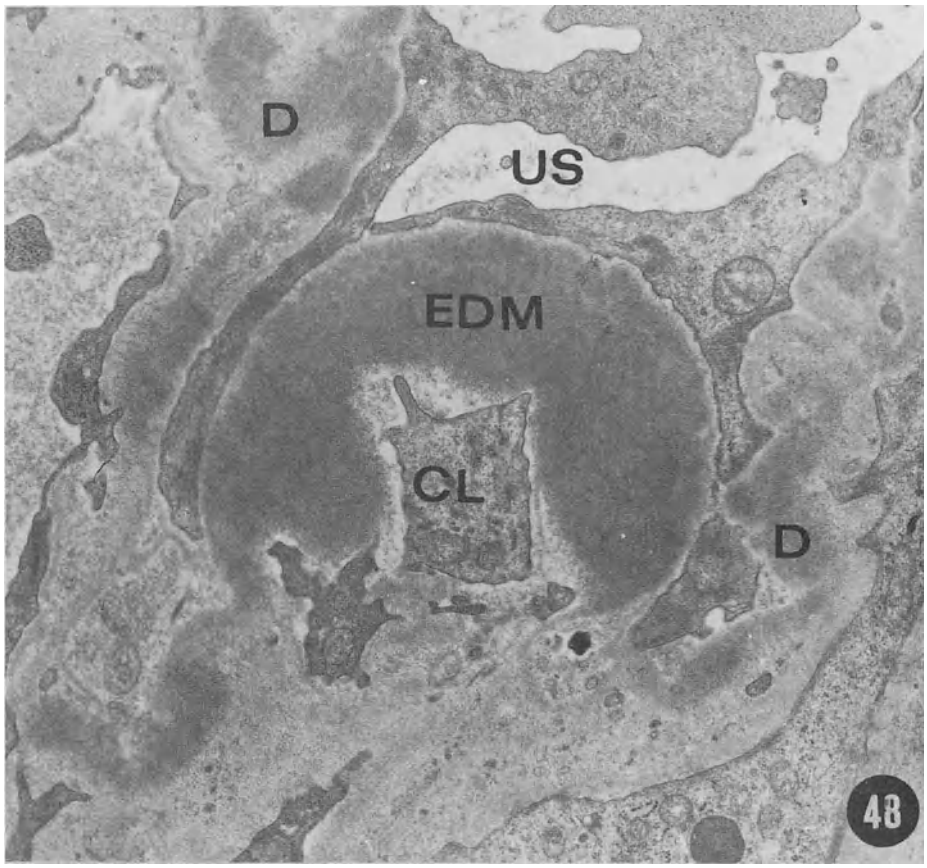

Fig. 6-48. Magnified view of one of the electron-dense masses (EDM). Deep electron-dense deposits (D) are widespread and present in almost all capillary loops. Lumen of the capillary (CL) and urinary space (US) are shown (UA + LC, ×21,000).

An indeterminate percentage of children and adults have idiopathic proteinuria or nephrotic syndrome and progress slowly to renal failure. Retrospective histological reevaluation in such patients has documented a new clinicopathological entity with various names (see the synonyms).

This lesion was first observed by Rich in 1957, in children with nephrotic syndrome and minimal or no change in the glomeruli who failed to respond to corticosteroid therapy. The pathological condition was not recognized until 1969, when Hayslett and colleagues observed renal insufficiency in a group of patients who were known to have lipoid nephrosis. In 1970, Churg and co-workers were able to isolate glomerular disease associated with sclerotic changes as a separate and definite entity among children with nephrotic syndrome. Since then, numerous individuals have recognized this condition and reported their results. This

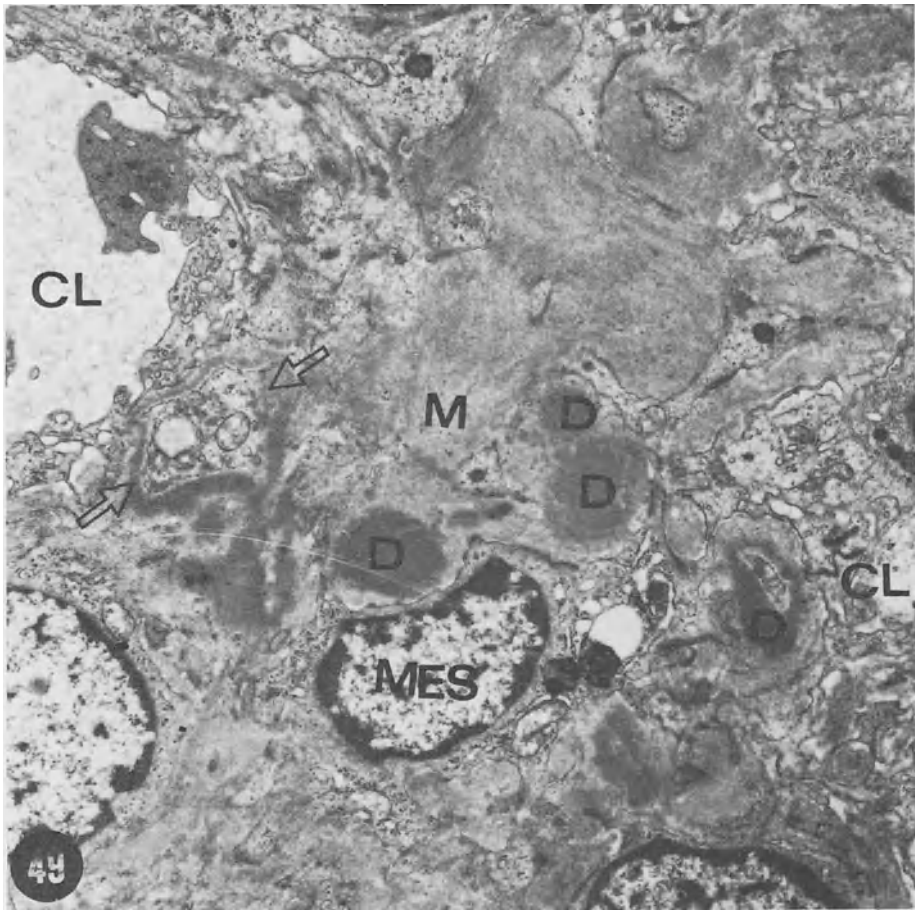

Fig. 6-49. Discrete electron-dense deposits (D) in the mesangium. Note mesangial cell (MES) and excessive matrix in the mesangium (M). Note capillary lumen (CL) and electron-dense deposits in the capillaries (arrows) (UA + LC, ×10,000).

pathological state is manifested clinically by mild to severe proteinuria, nephrotic syndrome, microscopic hematuria, hypertension, slowly progressive renal failure, and no response to corticosteroid therapy. Although this lesion was first described in children, and appears to be common in children, it is increasingly recognized in adults. In our own limited observation, seven of nine patients were adults; all of these patients had proteinuria, two-thirds of them had impaired renal function, and slightly more than half of the patients had hypertension. Follow-up after a variable period of 6 to 84 months revealed increased proteinuria and renal failure in all patients (for details see Mandal *et al.*, 1976).

Light microscopy histology appears consistent in all series and therefore can be considered as the most reliable aid for the diagnosis. It consists of focal segmental and focal global glomerular sclerosis (Figs. 6-52 to 6-53), intraluminal

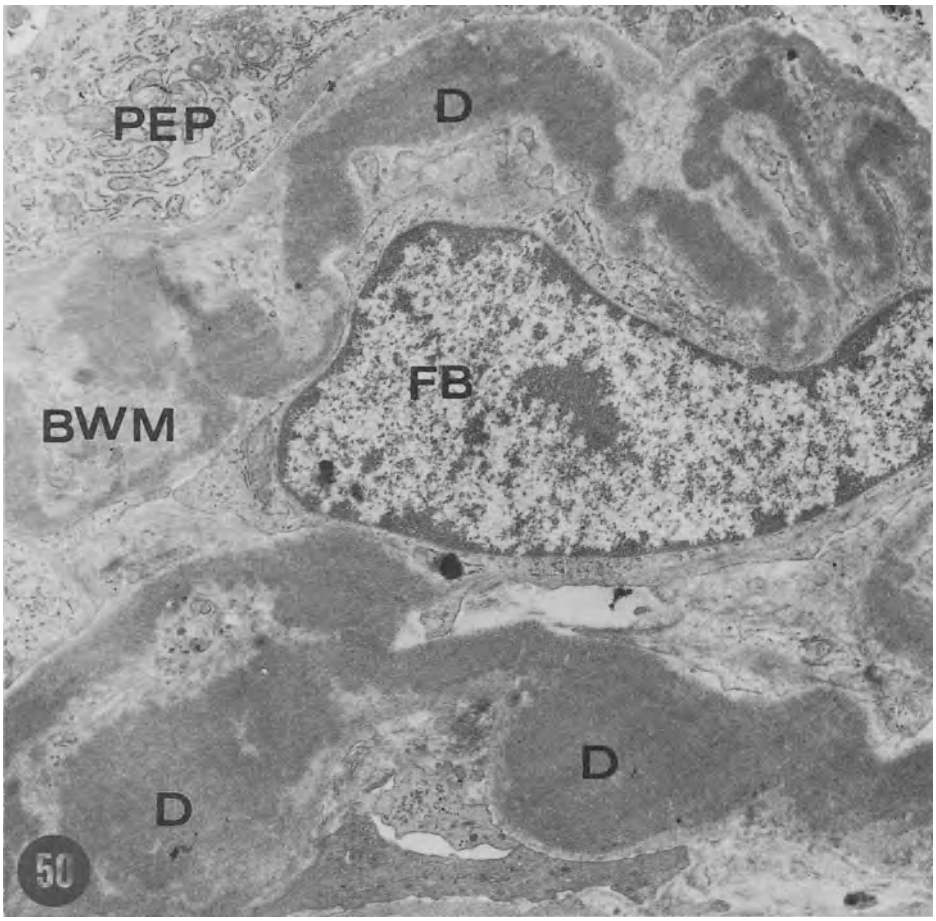

Fig. 6-50. Massive electron-dense deposits (D) throughout the tubular basement membranes, and Bowman's membrane (BWM). Fibroblast (FB) is seen in the interstitial space. Parietal (Bowman's) epithelial cell (PEP) is shown (UA + LC, ×20,000).

hypereosinophilic and PAS-positive material (Fig. 6-54), with remaining glomeruli being normal (Fig. 6-52), mild to severe tubular atrophy, interstitial infiltrates of mononuclear cells, and fibrosis (Fig. 6-52). A note of caution is that the light microscopy findings of focal glomerular sclerosis (sclerosing glomerulonephritis) may be confused with those of focal proliferative glomerulonephritis. The former can be distinguished by (1) absence of hypercellularity (Fig. 6-53), (2) segmental sclerosis at the vascular pole of the glomerulus (Fig. 6-53), (3) intraluminal hyaline material, especially within the sclerotic portion of the glomerulus (Fig. 6-54), and (4) intracapillary foam cells (Fig. 6-54).

The electron microscopy study is not nearly as convincing as the light microscopy study and is rather controversial; for example, Hyman and Burkholder (1973) found massive subendothelial electron-dense deposits in many patients, whereas Nagi *et al.* (1971) and Matalon *et al.* (1974) did not find electron-dense deposits in any of their patients. The negative findings for electron-dense deposits may be false negatives caused by the focal nature of this disease and by the sampling error of EM study. Furthermore, failure to find deposits in UA + LC-stained sections does not exclude the existence of deposits, since thin sections

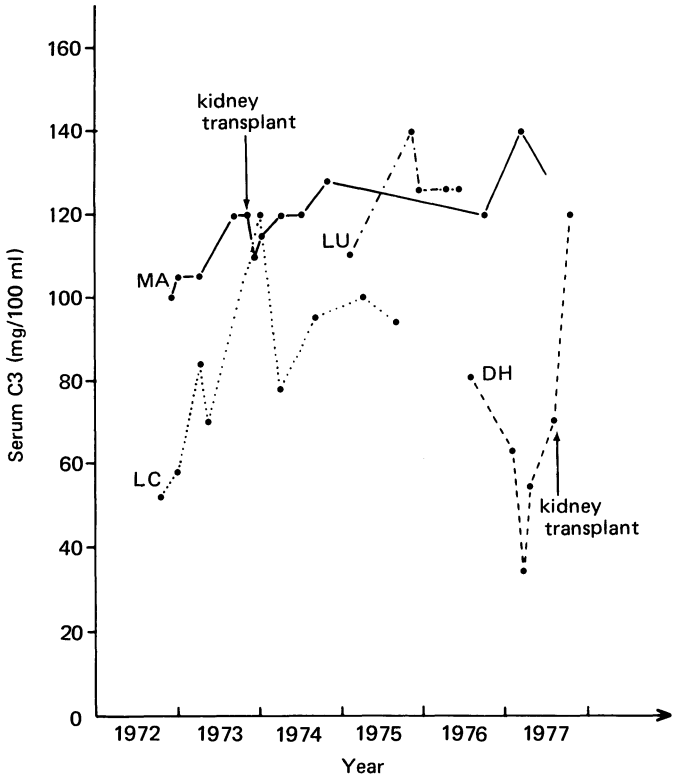

Fig. 6-51. Serial C3 measurements in patients with mesangioproliferative glomerulonephritis. Normalization or near normalization of serum C3 after kidney transplantation in two patients is shown.

stained with PAMS in the author's laboratory have demonstrated numerous silver-positive deposits. In our own experience, massive subendothelial deposits of poor electron density, small intramembranous and extramembranous deposits with occasional spiky appearance of the GBM, endocapillary foam cells, and sheets of GBM-like material encompassing glomerular capillaries are considered helpful EM features (Figs. 6-55 and 6-56) to supplement the LM diagnosis.

Hyman and Burkholder (1973) and Habib (1973) believe that the intraluminal hyaline materials (hypereosinophilic and PAS-positive materials) seen by LM are equivalent to the deposits observed via EM. We are in complete agreement with these authors. Habib described two histopathological variants of focal glomerular sclerosis: focal and segmental hyalinosis, and focal glomerular obsolescence. One of the two variants may be found in some patients, but both variants are observed in most patients. In the author's experience, focal and segmental sclerosis and hyalinosis appear to be common in adults.

There are some differences observed in the mode of presentation and progression of the disease process in children and adults. These are listed in Table 6-12. Thus, in the adult series (Hyman and Burkholder, 1973), 70% of the patients

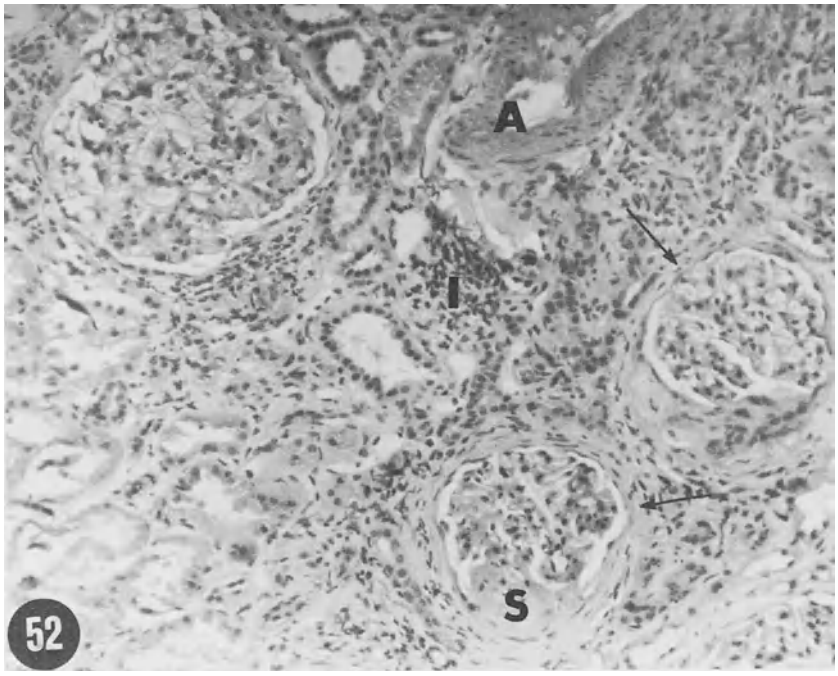

Fig. 6-52. Sclerosis (S) at the vascular pole of a glomerulus. The other two glomeruli appear essentially normal except for slight pericapsular fibrosis (arrow) in one, which is also visible in the glomerulus with vascular pole sclerosis. Patches of cellular infiltration in the interstitium (I) and a few tubules are seen. A thickened small artery (A) is shown (H & E, ×120).

developed hypertension and renal insufficiency in 9 years from the onset of the disease process. In follow-up studies of 88 children (Habib, 1973) ages 3 months to 22 years (average 7 years), it was found that 28% of the patients died or had been subjected to hemodialysis treatment. Hypertension and impairment of renal function occurred in only a very small percentage.

Mechanisms of Focal Glomerular Sclerosis

Recognition of focal glomerular sclerosis as a separate entity is still controversial. There is no direct evidence to contradict the belief that focal glomerular sclerosis is a distinct disease. Although the evidence in favor of this disease process is mostly circumstantial and anecdotal, an experimental study lends strong support to this side of the argument. The evidence is as follows: (1) The segmental sclerosis and hyalinosis appear to progress. (2) The hyalinosis appears too conspicuous to be explained by mere scarring of a healed lesion. (3) The finding of progressive changes in the tubules, interstitium, and arterial vessels which accompany glomerular lesions reinforces the idea of a definite pathological entity. (4) The EM findings, comprised of electron-dense deposits, endocapillary

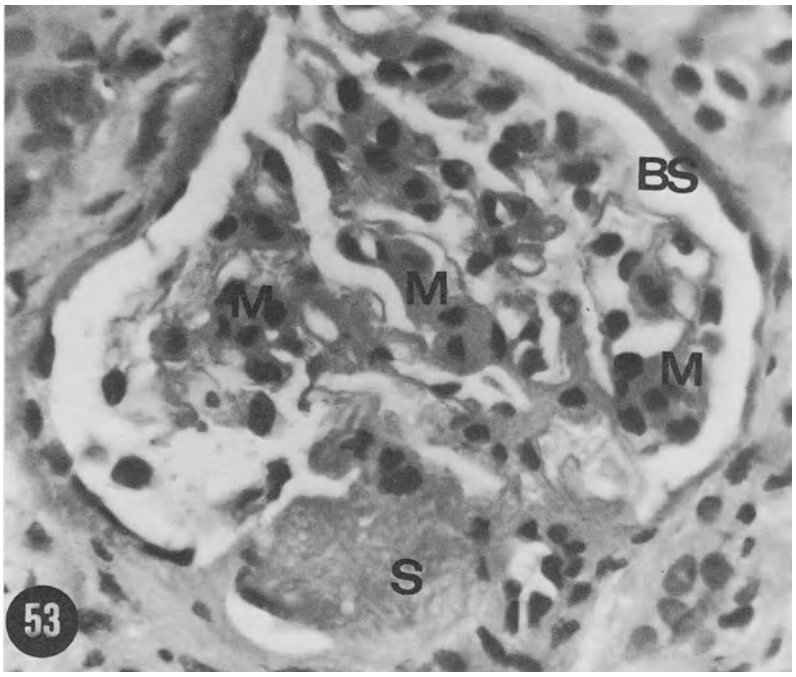

Fig. 6-53. Sclerotic material (S) at the vascular pole of the glomerulus is clearly discerned. Slight increase of matrix in the mesangium (M) is shown; contraction of the glomerular tufts and enlargement of Bowman's space (BS) are apparent (PAS, ×400).

Table 6-12
Differences in Clinical Presentations and Progression between Children and Adults with Focal Glomerular Sclerosis

Clinical features	Children	Adults
Microhematuria	Common	Uncommon
Asymptomatic proteinuria	Uncommon	90% or more
Nephrotic syndrome	70–80%	10% or less
Hypertension	Rare	50–60%
Progressive renal failure	10–20%	50–60% or more
Response to corticosteroid therapy	Slight or none	Slight or none
Histological variants		
Focal and segmental hyalinosis	Incidence unknown	Incidence unknown
Focal glomerular obsolescence	Incidence unknown	Incidence unknown
Survival (without dialysis and transplantation)	More than 10 years in most cases	Undetermined

foam cells, and sheets of GBM-like material, are supportive of a definite pathological process. (5) The progressive increase in proteinuria and the decrease in renal function appear to comprise a hallmark of an active pathological process. (6) Only a small percentage of patients among the large number of referrals for asymptomatic proteinuria are found to have focal glomerular sclerosis. (7) Reports of recurrence of the disease in transplanted kidneys certainly support a true disease entity. (8) Experimental studies attempting to produce a model of focal

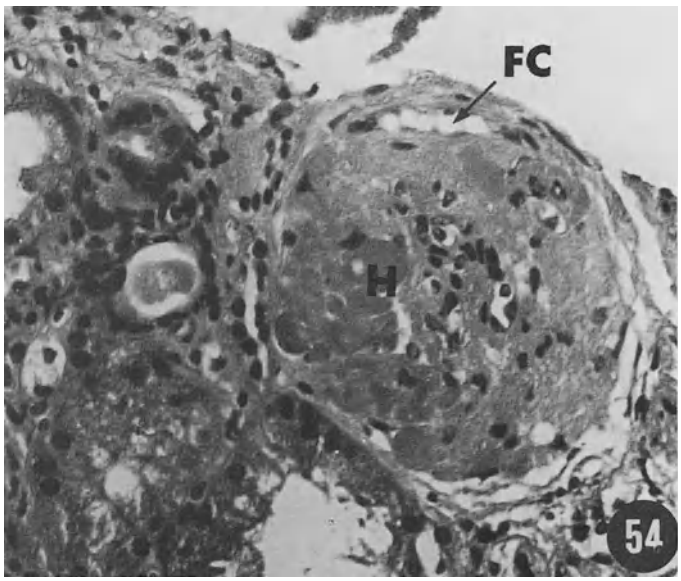

Fig. 6-54. Completely sclerotic glomerulus with still-discernible intraluminal hyaline material (H). Intracapillary foam cell (FC) is shown (PAS, ×100).

glomerular sclerosis have concluded that this lesion may be secondary to epithelial cell injury, a conclusion that is supported by the observations of Grishman and Churg (1975) from their studies on human renal biopsies. These authors found that some cases of focal glomerular slcerosis are associated with severe damage to podocytes caused by drugs, infection, or unknown factors. This, then, may contribute to the development and progression of glomerular lesions in sclerosing glomerulonephritis.

There is an observation which seems to contradict the notion that focal

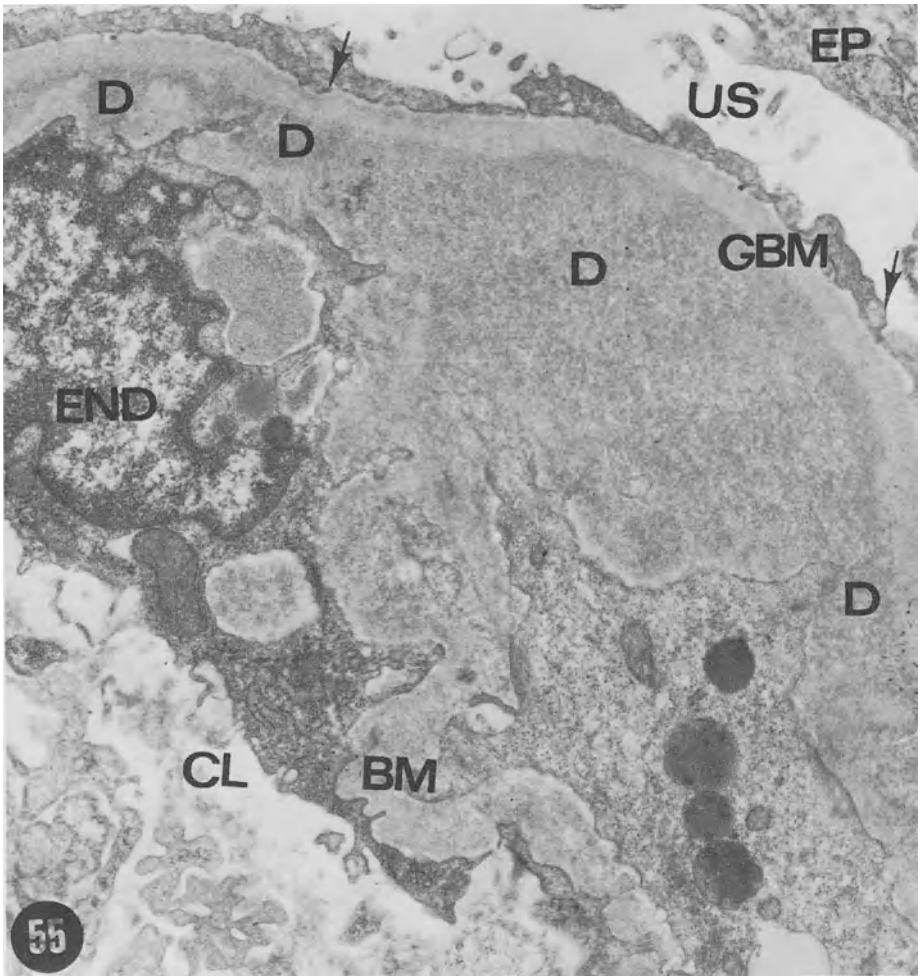

Fig. 6-55. This glomerular capillary reveals essentially normal basement membrane (GBM), subendothelial electron-dense deposits (D), excessive basement membrane-like materials (BM), and fusion of foot processes (arrows). Lumina of the glomerular capillary (CL), urinary space (US), and epithelial cell (EP) can be seen (UA + LC, ×18,000).

glomerular slcerosis is a separate disease entity. In a study of 334 renal biopsies in England, only 57 biopsies revealed segmental lesions. These 57 biopsies were reviewed by two independent observers, and an agreement on focal glomerular sclerosis was obtained in less than 10% of the biopsies.

VIRAL INFECTIONS AND GLOMERULONEPHRITIS

Viral infections and renal disease can be divided into three separate categories: (1) abnormal urinalysis in acute viral infections, (2) acute renal failure

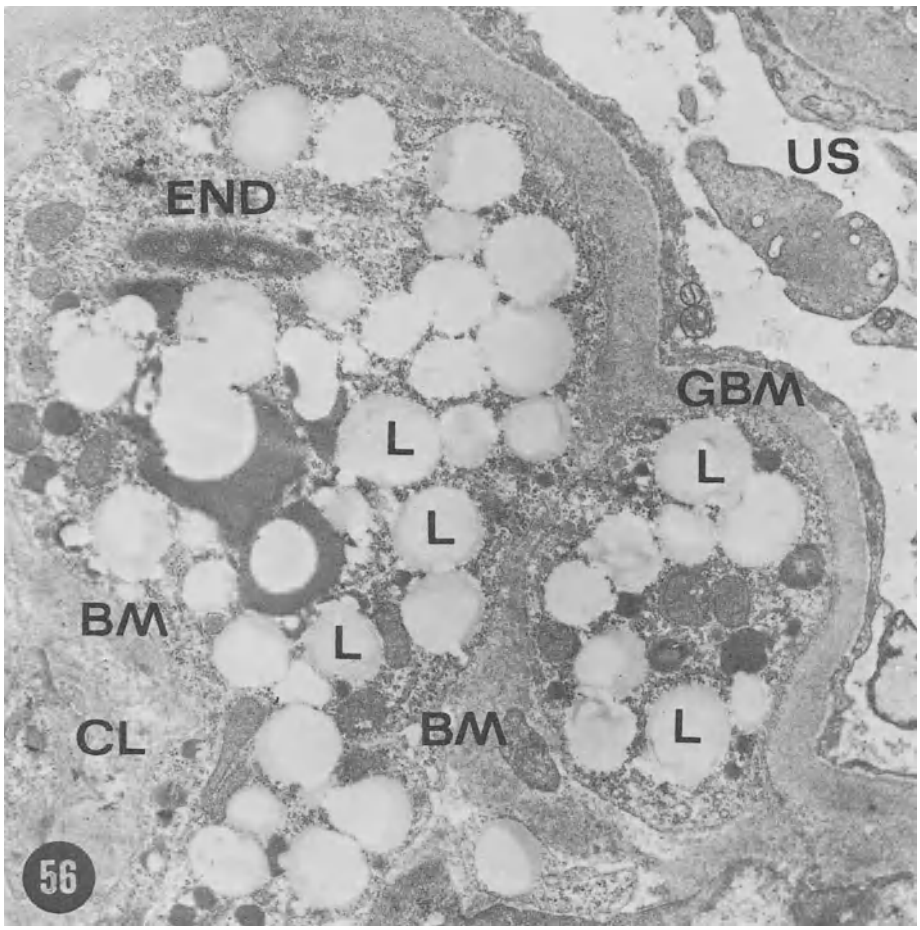

Fig. 6-56. Endocapillary foam cell formed from lipid droplets (L) within the endothelial cell (END). The glomerular basement membrane (GBM) appears normal. The capillary lumen (CL) has been compromised by excessive basement membrane-like materials (BM). Urinary space (US) is shown (UA + LC, ×16,000).

Table 6-13
Viruses Associated with Manifestations of Renal Diseases

Virus	Major disease	Renal manifestations
Mumps	Parotitis	Hematuria, proteinuria, viruria, decreased creatinine clearance
Coxsackie B	Aseptic meningitis, pancarditis	Viruria, decreased PSP excretion, focal glomerulonephritis with interstitial nephritis (autopsy)
Echo	Aseptic meningitis, pharyngitis	Hematuria, proteinuria, acute proliferative glomerulonephritis (biopsy)
Adeno-	Pharyngoconjunctival fever	Hematuria
Vaccinia	Primary vaccination	Diffuse necrotizing glomerulonephritis
Cytomegalo-	Cytomegalic inclusion disease in infants and children, cytomegalo-viral infection in adults (due to immuno-suppression)	Viruria, inclusions in urinary sediment
Measles	Exanthem	Viruria
Infectious hepatitis	Hepatitis	Proteinuria, hematuria
Hepatitis-associated antigen	Polyarteritis nodosa	Diffuse proliferative glomerulonephritis

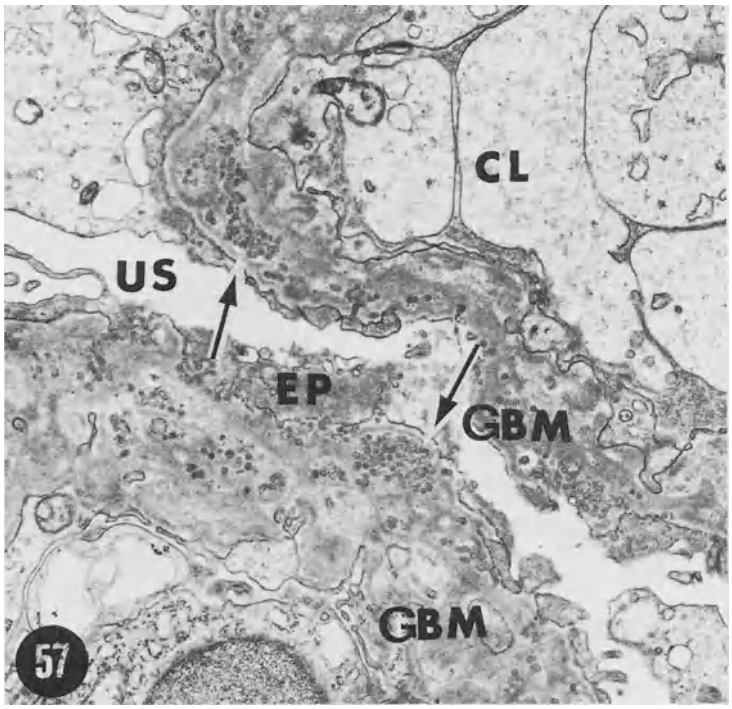

Fig. 6-57. Clusters of inclusion bodies (arrows) within the glomerular basement membranes (GBM) in serial biopsies of two patients with nephrotic syndrome. Lumen of the capillary (CL) and urinary space (US) are shown (UA + LC, ×10,000).

associated with acute viral infections, and (3) progressive renal disease in which viral infection has been suspected but not confirmed.

Viruses have been implicated in a variety of renal parenchymal diseases, although in only a few instances has renal biopsy been performed to document the renal lesion. The evidence for renal disease caused by a virus is circumstantial in most instances; seldom, if ever, has a culture of renal tissue grown viruses.

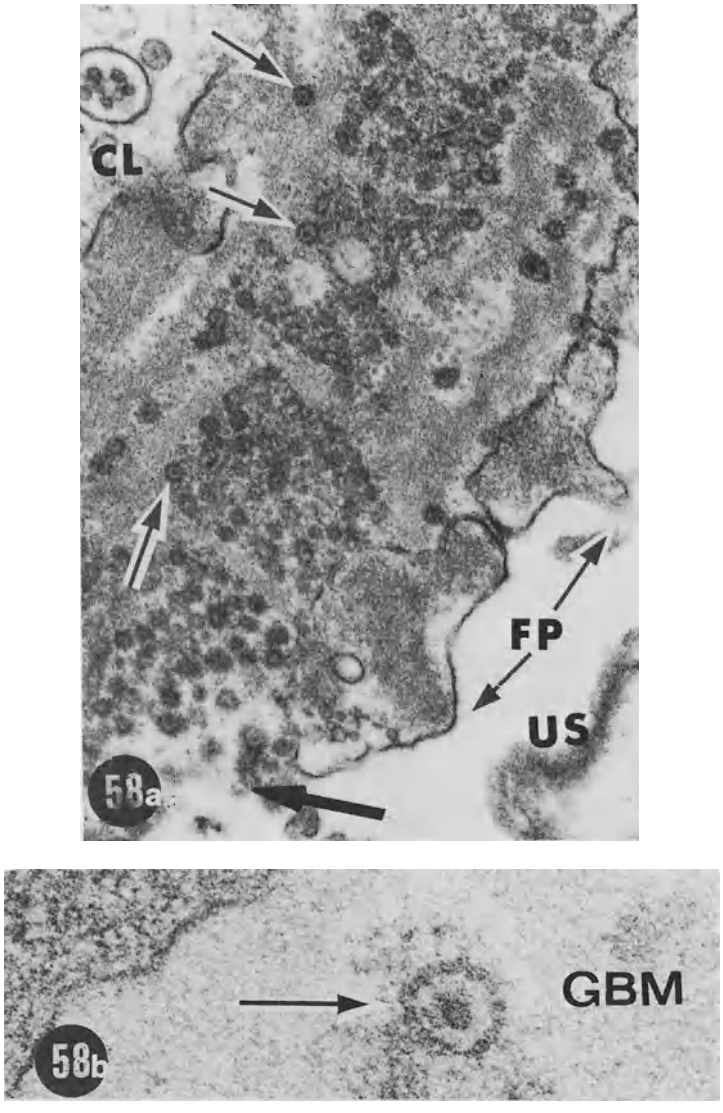

Fig. 6-58. (a) Magnified view of the clusters shows particles which demonstrate a central electron-dense core and spiked membrane (arrows). Foot processes (FP), urinary space (US), and lumen of the capillary (CL) are shown (UA + LC, ×24,000). (b) Note a viral particle (arrow) in the glomerular basement membrane (GBM) (UA + LC, ×200,000).

The manifestations may be mild and detected by routine urinalysis, or severe, associated with a florid picture of acute glomerulonephritis or acute renal failure. A list of viruses, major viral diseases, and the renal manifestations is given in Table 6-13. Microtubular structures have been observed within the endothelial cells of glomerular capillaries via EM in several types of glomerulonephritis, the most notable of which is lupus glomerulonephritis (Fig. 6-19). Others include membranous GN and rapidly progressive GN.

We have found viruslike inclusions within the glomerular basement membranes in three successive biopsies from two adults with membranous glomerulonephritis. The inclusion bodies were found mostly in clusters, as well as single and in pairs, throughout the basement membranes in all capillary loops in all three biopsies. These inclusion bodies were almost always spherical, consisting of a central electron-dense core and surrounded by spiked membrane (Figs. 6-57 and 6-58). The mean diameter of these inclusion bodies was 89 nm (range 46–146 nm). The average number of clusters changed little between the first and subsequent biopsies; however, the number of inclusion bodies per cluster appeared greater in the follow-up biopsies. An additional biopsy in January 1978, from one of the two patients, revealed a wider spread of the inclusion bodies into the endothelial cells, epithelial cells, and even the peritubular capillaries. The ultrastructural features and the diameters of these inclusion bodies are consistent with myxoviruses such as influenza, mumps, and measles. From this and similar observations made by other investigators, viruses may be implicated in the pathogenesis of membranous glomerulonephritis. Also, in view of the prevalence of viral infection and the lack of knowledge concerning the etiology of idiopathic membranous GN, these limited studies should stimulate further investigation to eliminate or confirm the proposed etiological role of viruses in membranous GN.

SUMMARY

1. Generally speaking, the common types of glomerulonephritis encountered in clinical practice are proliferative, membranous, and mesangioproliferative. The idea of considering focal glomerular sclerosis or sclerosing glomerulonephritis as a separate entity is still nebulous; however, it is being increasingly studied.

2. From the standpoint of clinical practice, diffuse glomerulonephritis can be separated with the aid of EM into (a) endocapillary proliferative GN, characterized by marked proliferation of endothelial and mesangial cells, severe PMN exudation, and typical "humps"; (b) endocapillary proliferative GN, characterized by mild proliferation of endothelial and mesangial cells, mild PMN exudation, and conspicuous subendothelial deposits; and (c) extracapillary proliferative GN, characterized mainly by epithelial cell proliferation, disruptive GBM changes with mild PMN exudation, and absent or inconspicuous electron-dense deposits.

3. The severity of clinical presentation seems to parallel the degree of histopathological change. Thus, the more severe the symptoms (e.g., oliguria or anuria, hypertension, gross hematuria) the more severe the histopathological changes may be. A typical "hump" or another glomerular lesion, e.g., necrosis or thrombosis, has generally been associated with more severe symptoms than mild endocapillary proliferative GN without a "hump," which tends to have milder symptoms.

4. Recovery rate is greater and sequelae are fewer in mild glomerular lesions without a "hump," necrosis, or thrombosis than in glomerular lesions associated with the latter changes. Recovery is much higher in those with a typical "hump" than those with an atypical "hump."

5. Lupus nephritis has been classified differently by different investigators. From the clinical standpoint, a division of minimal, proliferative, and membranous types appears to be convenient. EM and IFM studies are mandatory in determining the difference since prognosis and effect of treatment would vary according to the histopathological category. The activity of the disease process can be assessed by a variety of LM or EM findings. In general, cellular proliferative change accompanied by large and deep subendothelial electron-dense deposits indicates an active process, whereas small discrete intramembranous or extramembranous deposits signify an inactive process.

6. Crescentic glomerulonephritis *per se* is the most serious type, especially when crescents occur in more than 50% of glomeruli. It has a lesser tendency to reverse and is generally resistant to treatment. The glomerular crescents involving 10 to 20% of glomeruli can be observed in endocapillary proliferative GN and in renal disease other than RPGN. The crescent formation to this extent is of no or less serious significance.

7. The mechanism or mechanisms of proliferative or necrotizing GN accompanied by the presence of thrombi in glomerular and extraglomerular capillaries without definite evidence of intravascular coagulation (e.g., TTP, HUS) are not yet fully understood. Consequently, the therapeutic approach is in abeyance for this group of diseases.

8. Membranous GN is easy to diagnose, especially in the advanced stage, even by ordinary light microscopy. However, we are ignorant of the etiology of this pathological process. The disease is slowly progressive, and treatment with therapeutic agents such as corticosteroids or immunosuppressives is of dubious benefit. The most promising aspect of membranous GN is the concept of staging. It is believed that routine EM study and staging of membranous GN would contribute to improvement of our knowledge about the natural history of this disease. It is hoped that the staging method will assist us in establishing the benefit of therapeutic agents in this disease.

9. Mesangioproliferative GN appears by LM study as lobular GN in most cases and as dense deposit disease in some others. However, the lobular type demonstrates, by EM, parallel GBM, and an endothelial cell is seen interposed between the parallel GBM. This finding has been interpreted variously as mes-

angialization or railroad (train track) appearance of the capillary loop. Subendothelial deposit is a common finding. These characteristics are consistent with type 1 MPGN. The type 2 MPGN demonstrates deposition of electron-dense materials which occupy two-thirds to three-fourths of the width of individual capillaries and appear in almost every capillary in all glomeruli. Since serum C3 is consistently low to very low in this type, the term *hypocomplementemic glomerulonephritis* may be rightly applied to this type of MPGN. Also, the denser and more widespread the deposits, the lower is the C3 observed. Serum C3 level varies but never normalizes. This type is believed to run a more rapid course than the other types, but this idea is not universally accepted. Dense deposit disease also is known to recur in the transplanted kidney. Although serum C3 has been reported to remain low after transplantation associated with high incidence of recurrence, normalization of serum C3 may occur, and this may possibly serve as an index against recurrence of the disease.

10. The clinical spectra associated with focal glomerular sclerosis vary; 5 to 10% of nephrotic children demonstrate this lesion, and 2 to 4% of adults with asymptomatic proteinuria, hypertension, and mild renal insufficiency are found to have it. The mechanism(s) and the natural history of this pathological process are poorly understood. Clinically, the disease progresses more rapidly in adults than in children, and, thus far, all attempts to halt the progression with therapeutic regimens have failed.

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7 Anatomic Pathology of the Kidney in Nephrotic Syndrome: Clinicopathological Abridgement

INTRODUCTION

Nephrotic syndrome is a clinical condition characterized by heavy proteinuria and manifested by edema. Daily loss of protein in the urine may vary, but a 24-hr protein loss exceeding 3.5 g on more than one occasion is the most important criterion used to establish the state of nephrotic syndrome. Accompanying but less important features are hypoalbuminemia and hyperlipidemia. These clinical and laboratory findings indicate renal parenchymal disease but fail to point toward the type of renal lesion.

By and large, nephrotic syndrome is the result of disease of the glomeruli, but it is not rare to see the nephrotic syndrome in patients with chronic tubulointerstitial diseases. Nephrotic syndrome occasionally occurs in severe (malignant) hypertension; whether or not nephrotic syndrome is a result of arterial vascular lesions or secondary glomerular changes, or both, has remained unsettled. Circumstantial evidence, such as a history of sore throat preceding an illness which appears to be an episode of acute glomerulonephritis (GN), symptomatic recurrent urinary tract infections, history of long-standing diabetes mellitus, severe hypertension, infection, or drug or food allergy, may predetermine the type of renal lesions. However, all attempts to guess renal pathology associated with nephrotic syndrome should be avoided.

It was mentioned in Part 1 of Chapter 2 that nephrotic syndrome, especially in adults, is an ideal indication for renal biopsy. Since the majority of patients seen in the practice of nephrology present with nephrotic syndrome, the knowledge gained via renal biopsy studies in nephrotic syndrome has helped to improve medical care significantly.

There is no longer any argument that renal biopsy study, with a few excep-

tions, is the major step in the evaluation and management of patients with nephrotic syndrome. The most important reason for this procedure is to avoid missing renal diseases which are reversible or potentially reversible. With the aid of renal biopsy study, it has been established that nephrotic syndrome is a clinical condition which may be caused by a variety of renal pathological states. These causes have been described in various ways by different authors.

For the purposes of clarity and ease in reading, we would like to present the spectra of renal diseases which have been found to be associated with nephrotic syndrome, i.e., glomerular diseases, tubulointerstitial diseases, vascular diseases, and a combination of glomerular, tubular, and vascular diseases.

Glomerular diseases are subdivided according to the frequency of observations in combined populations of children and adults:

1. Idiopathic nephrotic syndrome or foot process fusion disease (lipoid nephrosis).
2. Idiopathic glomerulonephritis: (a) membranous glomerulonephritis; (b) mesangioproliferative or mesangiocapillary (lobular) glomerulonephritis; (c) sclerosing glomerulonephritis or focal glomerular sclerosis.
3. Glomerulonephritides of diverse etiology. Notable among these are (a) lupus membranous glomerulonephritis; (b) unhealed poststreptococcal glomerulonephritis; (c) syphilitic glomerulonephritis; (d) glomerulonephritis of sickle cell anemia; (e) membranous glomerulonephritis associated with unilateral or bilateral renal vein thrombosis; (f) membranous glomerulonephritis associated with malignant neoplastic conditions, e.g., carcinoma, lymphoma, leukemia.
4. Glomerulosclerosis of diabetes mellitus.
5. Foot process fusion disease associated with malignant neoplasms, e.g., Hodgkin's disease.
6. Glomerular diseases induced by drugs, e.g., tridione, penicillamine, heroin, bismuth.

Tubulointerstitial diseases:

1. Chronic pyelonephritis (chronic interstitial nephritis).
2. Hereditary nephritis.
3. Chronic transplant rejection.

Vascular diseases:

1. Malignant hypertensive renal lesions.

Combination of glomerular, tubular, and vascular diseases:

1. Amyloidosis.
2. Congenital nephrotic syndrome.

Of the pathological processes enumerated, those that are reversible include (1) idiopathic nephrotic syndrome, (2) syphilitic glomerulonephritis, and (3) drug-induced glomerular diseases. The potentially reversible processes are (1) idi-

opathic membranous GN, (2) lupus membranous GN, and (3) renal vein thrombosis.

IDIOPATHIC NEPHROTIC SYNDROME

SYNONYMS. Lipoid nephrosis, foot process fusion disease, epithelial cell disease, "nil" disease, minimal lesion disease.

Idiopathic nephrotic syndrome is the most common (80–90%) type of nephrotic syndrome in young children 6 months to 5 years of age. It is not as common as once thought in older children and adolescents, and comprises only 20 to 30% of cases of nephrotic syndrome in adults. Both the preceding event and the pathogenetic mechanism for this disease process are currently unknown. Comprehensive studies using electron microscopy (EM) and immunofluorescence microscopy (IFM) have failed to define a mechanism for the widespread fusion of glomerular foot processes in idiopathic nephrotic syndrome. IgE deposition in the glomeruli had been implicated as a possible mechanism by one group of investigators but this was not confirmed by others.

Pathology

None of the proposed terms is appropriate to denote the pathology. However, foot process fusion disease or epithelial cell disease is justified since the principal pathological finding involves epithelial cell foot processes. The term lipoid nephrosis is considered to be the least appropriate since it merely signifies degenerative changes of tubular epithelium by lipids. It must be remembered that lipid droplets in the tubules are a consistent finding in hyperlipidemia and do not constitute, per se, a pathological state. Until a better term is found, it seems advisable to use idiopathic nephrotic syndrome or any of the synonyms as a working diagnosis.

Glomeruli appear essentially normal by light microscopy (Fig. 7-1).* The observation of the slight increase in mesangial cellularity often reported is an overinterpretation. We have found the glomeruli to be absolutely normal by examination with the oil immersion lens. Electron microscopy shows the consistent abnormality to be generalized fusion of the foot processes forming a smeary layer over the basement membrane (Figs. 7-2 to 7-4). The epithelial cell is normal or hyperactive, the latter characterized by the presence of numerous and large-sized mitochondria, many rough-surfaced endoplasmic reticula, Golgi complexes, ribosomes, and a large or bifid nucleus (Fig. 7-3). The glomerular basement membrane (GBM) is essentially normal. Slight irregularities on the endothelial aspect of the basement membrane in some glomerular capillaries and a slight

* Figures 7-1 and 7-2 are from the renal biopsy of a 6-year-old white male with recurrent nephrotic syndrome. The initial episode of nephrotic syndrome responded to cyclophosphamide but subsequent episodes responded to corticosteroid (prednisone). For details see Chapter 13.

increase in mesangial matrix are not uncommon. Retrospectively, the slight GBM changes are found in patients who have received corticosteroids for weeks before biopsies were obtained. The cellularity is normal and polymorphonuclear leukocyte (PMN) exudation is absent. It must be mentioned that widespread fusion of foot processes, i.e., involving the peripheral as well as centrilobular portions of the individual capillaries, is seldom, if ever, observed in any condition other than idiopathic nephrotic syndrome. The tubules are essentially normal although lipid droplets in the cells and slight separation of the tight junctions of the cells are often observed.

There are several intriguing questions concerning idiopathic nephrotic syndrome which have not been answered. What causes the fusion of the foot processes and how does this fused epithelial cell foot process produce massive proteinuria? How does glucorcorticosteroid exert beneficial effect? What is the cause of recurrent or relapsing idiopathic nephrotic syndrome and how does cyclophosphamide affect this clinicopathological problem?

Argument still exists as to whether or not the fusion of foot processes is an effect of heavy proteinuria, as supported by the following facts: (1) Intravenous administration of serum albumin into normal dogs is followed by proteinuria and fusion of glomerular epithelial foot processes. (2) Serial biopsy studies have demonstrated restoration of normal foot processes, which coincides with the disappearance of proteinuria in these experimental dogs.

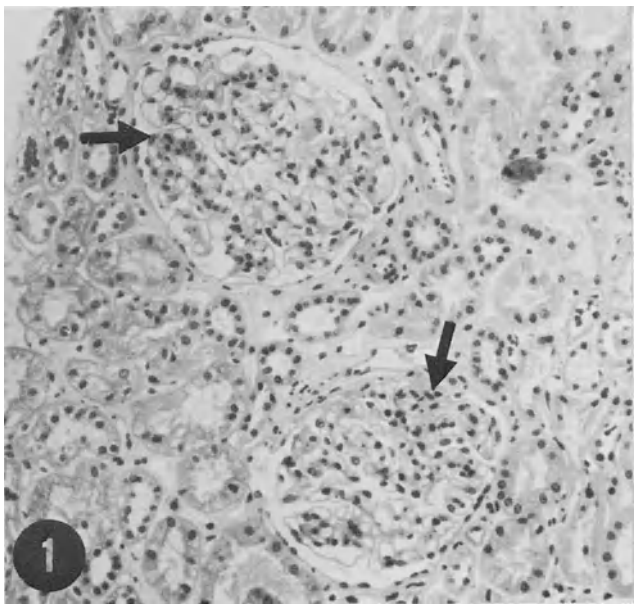


Fig. 7-1. Tubules and interstitium appear normal. Glomeruli, although reported to be normal, appear to have mild hypercellularity (arrows) (H & E, ×80).

It has remained a most difficult task to explain the beneficial effect of corticosteroid despite the complete absence of evidence of immunological involvement in this pathological process. The benefit cannot be explained simply by means of a diuretic effect since restoration of the normal morphologic appearance of the foot processes has been observed along with clinical remission of the nephrotic syndrome. Even so, the pathogenesis of recurrence of the syndrome and its response to immunosuppressive drugs, e.g., cyclophosphamide, are completely unknown.

Natural History

The natural history of idiopathic nephrotic syndrome is reasonably well established. Follow-up studies by several groups of investigators are in agree-

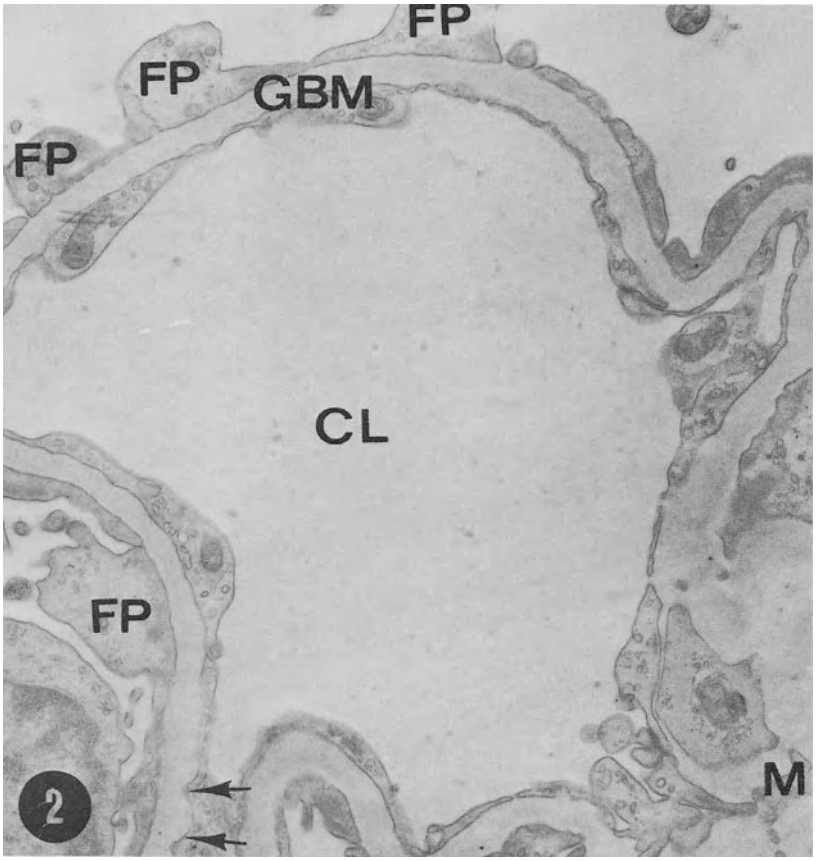


Fig. 7-2. The glomerular capillary reveals (1) normal glomerular basement membrane (GBM) with the exception of slight endothelial irregularities (arrows), (2) fusion of foot processes (FP) for the most part, and (3) slight increase of matrix in the mesangium (M). Lumen of the capillary (CL) is shown (UA + LC, ×10,000).

ment. The following is a summary of some of their observations:

1. The vast majority of children less than 5 years of age undergo complete and permanent remission with corticosteroid therapy. Children older than 5 years of age, adolescents, and adults may not always do so.
2. A small percentage (approximately 10%) of children have one or more recurrences of nephrotic syndrome.
3. Despite recurrent episodes, no children develop renal failure.
4. Serial biopsy studies have failed to demonstrate GBM thickening, disruption, or changes suggestive of membranous GN. However, it is not uncommon to see a slight increase in mesangial matrix.
5. Death during the nephrotic stage is most often due to infection. There is no evidence to indicate renal failure as a cause of death.
6. Oliguria and azotemia may occur in the nephrotic stage due to contracted

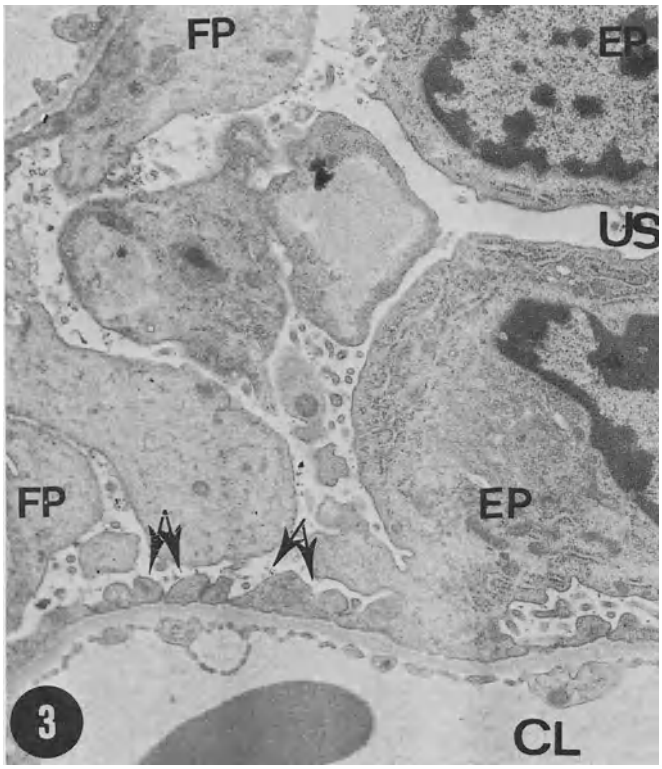


Fig. 7-3. In one glomerular capillary the foot processes are partly discrete (arrows), while in the other the foot processes are fused (FP). Epithelial cells (EP) appear active. Glomerular basement membrane appears normal. Capillary lumen (CL), and urinary space (US) can be seen (UA + LC, ×7500). From the renal biopsy of a 2-year-old white female with corticosteroid-responsive relapsing nephrotic syndrome.

extracellular space. It is a notable fact that diuresis occurs promptly following administration of corticosteroids and that shortly thereafter azotemia disappears.

7. It is now believed, based on serial renal biopsy studies, that focal glomerular sclerosis and not lipoid nephrosis is the cause of persistent nephrotic syndrome. The failure to recognize this pathology from the very beginning is because (a) focal glomerular sclerosis appears to involve the corticomedullary glomeruli before cortical glomeruli, which means that a few cortical glomeruli found in the small biopsy piece may not have demonstrable lesions; and (b) EM study does not aid significantly in the diagnosis of focal glomerulosclerosis. Therefore, it is clear that normal cortical glomeruli in the initial biopsies of patients with resistant or recurrent idiopathic nephrotic syndrome which was thought to be caused by lipoid nephrosis are now considered to be a false positive observation.

IDIOPATHIC MEMBRANOUS GLOMERULONEPHRITIS

Idiopathic membranous glomerulonephritis is the most common cause of nephrotic syndrome in the adult (greater than 21 years of age) population, accounting for 50 to 60% of nephrotic syndrome in adults and no more than 5% in young children, and occurring only rarely in infants.

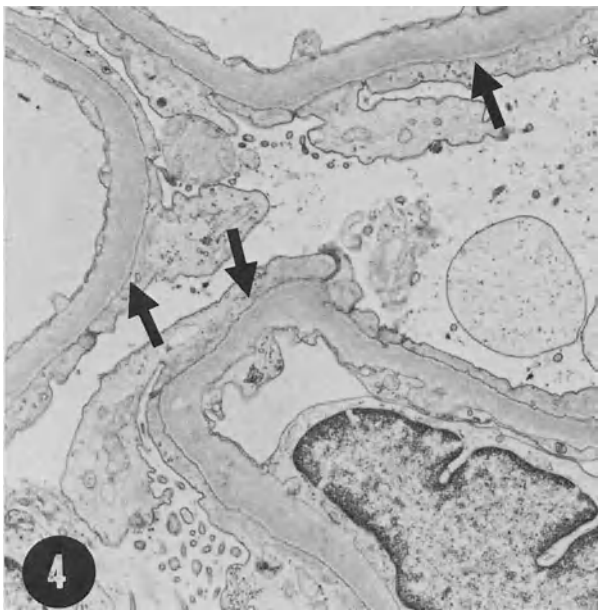


Fig. 7-4. The consistent abnormality in the glomerular capillaries is the generalized fusion of foot processes (arrows). The glomerular basement membranes are essentially normal (UA + LC, ×6000). From the renal biopsy of a 26-year-old white male who presented with nephrotic syndrome and had a complete response to corticosteroid therapy.

Pathogenesis

Pathogenesis is unknown, although low-grade antigenic stimulus for a prolonged period and slow formation of immune complexes have been postulated. Since GBM changes in idiopathic membranous GN resemble those induced by drugs, e.g., penicillamine, and those in secondary syphilis and "two-shot" serum sickness, it is imperative to consider the aforementioned possibilities as a cause of membranous GN.

Pathology

The pathology has been discussed in Chapter 6. In this chapter, the progression of the pathologic process and how the anatomic pathology of the kidney relates to the clinical syndrome are described. Because of the similarities of idiopathic membranous glomerulonephritis to membranous glomerulonephritis induced by drugs and that associated with renal vein thrombosis, the relationships between these and membranous glomerulonephritis are also discussed.

Although the severe changes in GBM, especially in the advanced stage of membranous GN, warrant a gloomy outlook for the patients, many patients have reasonably good lives. There are many difficulties in establishing the natural history of the histopathological process and the linear relationships between the extent of histopathological damage and severity of clinical manifestations. Notwithstanding the obstacles to an understanding of this clinicopathological syndrome, the author intends to outline some of its established characteristics:

1. The pathological process appears to undergo a progression that is manifested by an increase in the spiking of GBM and a decrease in the number and electron density of the deposits. It must be remembered, however, that there are not enough studies to indicate conclusively that stage 1 membranous glomerulonephritis progresses to stage 4 membranous glomerulonephritis. This will not be accomplished until a prospective study of the serial biopsies from individual stages of the disease process is completed.
2. It is well known that spontaneous remission of proteinuria occurs in 25 to 33% of the total number of patients. The remission is unrelated to the staging of the disease and may last for many years.
3. Renal failure occurs in 25 to 33% of patients with unremitting nephrotic syndrome after a variable period of 3 to 19 years. In one series, the average duration from first observation of nephrotic syndrome to appearance of uremia was 2.7 years.
4. Effectiveness of corticosteroid therapy in ameliorating proteinuria or retarding histopathological progress is subject to controversy.
5. There is some agreement among investigators that in stages 1 and 2 of membranous GN, clinical remission and histological reversibility can be expected following corticosteroid therapy.

6. Although the preceding may be true, there are no data to suggest that all patients who undergo clinical remissions will exhibit histological reversibility.

7. The cause for fluctuations in the severity of proteinuria, even with unchanged GBM damage, is unknown. It is generally believed that sclerosis in the advanced stage of the pathological process decreases the glomerular filtration rate (GFR), which consequently results in decreasing proteinuria. This notion is supported by the observations of sequential biopsy studies and their correlation with proteinuria. Hatta (1972) has observed that the continuation of deposition of new immunoprotein at the epithelial side of the basement membrane and injury and destruction of the basement membrane seem to correlate with the increase of proteinuria, and that the decrease of new deposits and the appearance of newly formed basement membrane at the epithelial side seem to correlate with the decrease of proteinuria. In any event, there is no doubt that GBM in stage 3 or 4 membranous GN has two layers, a thin layer with attached endothelial cells that resembles normal GBM and a thick and raggedy or spiky layer with fused epithelial cell that is unlike normal GBM and appears to be made up of fading deposits. (See Figs. 7-5 and 7-6, which are from the renal biopsy of a 37-year-old white male who presented with nephrotic syndrome. He gave a history of occa-

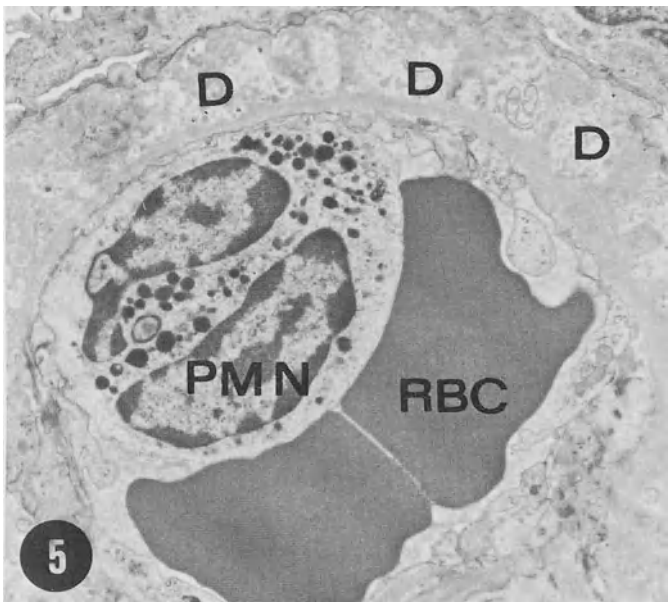


Fig. 7-5. Stage 3 membranous glomerulonephritis. The glomerular basement membrane demonstrates two distinct layers, an inner thin, normal-appearing layer and an outer wide and spiky layer. The electron-lucent areas are reminiscent of electron-dense deposits (D). Margination of a neutrophil leukocyte (PMN) to glomerular basement membrane is seen. Red blood cells (RBC) are shown (UA + LC, ×4600).

sional work with hydraulic jackhammers. Renal vein thrombosis was suspected, and was confirmed in the right renal vein by selected right renal venography.)

Idiopathic membranous glomerulonephritis resembles lupus membranous glomerulonephritis, membranous GN induced by drugs, syphilitic membranous GN, membranous GN associated with renal vein thrombosis, and membranous GN associated with malignant neoplastic conditions. Lupus membranous GN may be differentiated by (1) the presence of segmental lesions, i.e., some normal appearing glomerular capillaries; (2) the presence of intramembranous and subendothelial electron-dense deposits, in addition to spikes and extramembranous deposits (see Fig. 6-17); (3) evidence of proliferation of endothelial and mesangial cells; and (4) confirmation of the histological diagnosis by the serological tests for SLE. Nephrotic syndrome may occur in the setting of treated lupus proliferative GN. Although in such instances the disease process is less active, as shown by the decrease or disappearance of subendothelial deposits, it is likely that spike and extramembranous deposits would be found in the GBM (see Fig. 6-18). This condition is called membranous transformation of proliferative GN, a case history of which has been cited in Chapter 12 (patient #6, S.P.). For details of mesangioproliferative GN and sclerosing GN, see Chapter 6.

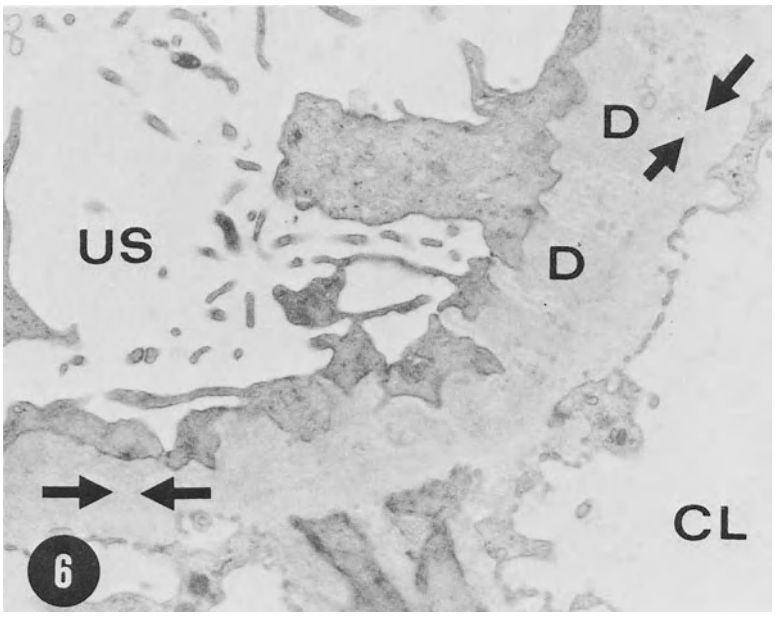


Fig. 7-6. Magnified view of a glomerular capillary basement membrane 1 year after the first biopsy (Fig. 7-5). Note the clear demarcation between two layers of glomerular basement membrane (opposing arrows) and light electron-dense deposits (D) in the outer spiky layer. Lumen of the capillary (CL) and urinary space (US) are seen (UA + LC, ×8400).

NEPHROTIC SYNDROME AND RENAL LESIONS PRODUCED BY DRUGS AND HYPERSENSITIVITY REACTIONS

A variety of drugs [e.g., trimethadione (tridione), paramethadione, probenecid, mercury compound (e.g., mersalyl), penicillamine, phenindione, gold salts, bismuth, and tolbutamide] and allergic, or hypersensitivity (e.g., bee stings, poison oak, and poison ivy), reactions are known to produce nephrotic syndrome. Renal biopsies have been performed in a few instances, although biopsy material has not been studied by EM or IFM in most of these cases.

In patients receiving one or more of these drugs, urinalyses every 2 to 3 weeks have been found to be a reliable index to determine the onset of renal disease. In those treated with trimethadione or paramethadione, renal lesions may not become manifest for 4 months to 5½ years, and thus periodic urinalysis is mandatory.

When the results of urinalysis are positive, dosage of the drug must be reduced. If these results become persistently abnormal, the drug must be completely withdrawn, whereupon nephrotic syndrome generally disappears. Nephrotic syndrome almost always recurs following reinstitution of trimethadione (paramethadione), probenecid, or penicillamine. The information is meager concerning renal pathology in drug-induced nephrotic syndrome, in particular with reference to reversibility or healing of renal lesion.

The cycles of disappearance and reappearance of nephrotic syndrome following withdrawal and reinstitution of some drug provide conceptual evidence of a reversible renal lesion, e.g., lipoid nephrosis. This is supported further by the response of the drug-induced nephrotic syndrome to corticosteroid therapy.

Renal Biopsy Studies in Drug-Induced Nephrotic Syndrome

Mercurial Preparations

Renal biopsies were studied in four patients in whom nephrotic syndrome was attributed to prolonged contact with mercury either on the job or as a result of mercury treatment for psoriasis. The light microscopy (LM) study revealed normal findings in three patients and membranous glomerulonephritis in one patient. EM study was done in the latter patient and was equivocal for membranous GN.

Penicillamine

There are numerous reports on the histopathological studies of penicillamine-induced renal disease. From all reports, it appears that penicillamine *per se*, or one of its metabolites, is toxic to kidney tissue.

In a series of 106 patients treated with penicillamine, about 9% developed proteinuria after 5 to 13 months of therapy. Proteinuria was severe enough to

cause nephrotic syndrome in half of these patients. The morphology in all reported studies includes membranous GN, focal glomerulonephritis, and crescentic glomerulonephritis. The clinical features and the morphology in the patient studied by the present author have been presented in Chapter 6. The author's patient did not have nephrotic syndrome; however, the morphology was consistent with membranous GN (see Figs. 6-38 to 6-40). Only rarely have uremia, hemoptysis and fatality following penicillamine therapy been reported. The author's patient developed irreversible uremia.

Gold Therapy

There are many reports concerning proteinuria and nephrotic syndrome following treatment with gold in rheumatoid arthritis. The glomerular changes suggestive of membranous glomerulonephritis have been described in most of these reports. The subject of "gold-induced renal lesion" raises several questions: (1) Is proteinuria caused directly by gold? (2) Does gold produce the aforementioned renal lesion and how does it induce the lesion? (3) Has it been denied that the renal lesion is a part of the immunological insult in rheumatoid arthritis? Another important question is whether nephrotic syndrome is due solely to membranous glomerulonephritis or to another renal lesion.

In answer to the first question, the evidence in support of gold as the direct cause of renal lesion is mostly circumstantial. The incidence of proteinuria is highly variable; for example, albuminuria was found in 13 of 900 cases (1.5%) of arthritis treated with gold for 4 years (Hartfall *et al.*, 1937); Silverberg and associates (1970) observed proteinuria in 7% (5 of 75) of rheumatoid arthritis patients treated with gold salts; Tornroth and Skrifvars (1974) encountered proteinuria in 70% (7 of 10) of rheumatoid arthritis cases treated with gold. In the last series, however, two additional patients with rheumatoid arthritis had proteinuria, although they did not receive gold treatment. In the Empire Rheumatism Council study, 4 of 90 patients being treated with gold and 3 of 95 untreated patients developed proteinuria. Some believe that gold is the causative factor and argue that the close relationship between the initiation of gold therapy and onset of proteinuria has been noted too often to be coincidental. Although contradictory evidence, i.e., proteinuria in patients who did not receive gold treatments, tends to refute the theory that proteinuria is induced by gold, the view cannot be dismissed. Despite the absence of direct evidence, a relationship seems to exist between gold therapy and proteinuria, especially since proteinuria disappears and reappears following withdrawal and reinstitution of gold, respectively.

In answer to the second question, the evidence that membranous glomerulonephritis is produced by gold is, once again, more circumstantial than not. The argument that membranous glomerulonephritis was not found in 2 of 12 rheumatics who did not receive gold is not a strong one. An important point in favor of gold as the causative agent is that identical membranous lesions were induced in rats after injection of gold salts by a group of investigators. However, there are doubts about these experimental results because they could not be reproduced

by other investigators. Even if gold is the causative agent, the mechanism by which gold salts may initiate the formation of immune complexes and the pathology of membranous GN are unknown. The nephrotoxicity of gold appears to be dose-related; proteinuria did not appear during weekly administration of 50 mg of sodium aurothiomalate but appeared when the dose of gold was doubled. High doses of gold administered to animals over a short period of time are known to produce extensive damage to the proximal tubules.

The following mechanisms of gold-induced renal lesions have been proposed: (a) Gold in some way damages a part of the glomerulus which then becomes antigenic and stimulates antibody production. The antibodies may then attach to the basement membrane, but, in this case, the immune deposits are usually located subendothelially. (b) Injury to the renal tubules by high doses of gold may initiate immune complex formation. In this context, demonstration of abundant deposits of immunoglobulins and complement in the epithelium and basement membrane of renal tubules is of some interest. This mechanism is unlikely because Silverberg and associates (1970) found no difference in the distribution and amount of gold present in the kidneys of patients treated with large amounts of gold and those without gold treatment. (c) It is known that gold is bound to plasma proteins. It has been suggested that gold may act as a hapten, stimulate the formation of antibodies to gold, and thence result in circulating immune complexes. This idea is contradicted by the failure of EM study to reveal the presence of gold in electron-dense deposits. Silverberg and associates did not find differences in the blood and urinary gold of the patients with nephrotic syndrome and those without it.

With regard to the third question, although the majority of data implicate gold as the cause of renal lesion in rheumatoid arthritis, it is difficult to rule out the role of rheumatoid arthritis in the pathogenesis of membranous GN. In the patients studied by Tornroth and Skrifvars (1974), rheumatoid factor was negative. It has been hypothesized that in the absence of positive rheumatoid factor, small-sized immune complexes would form which penetrate the basement membrane and cause diffuse injury.

Whatever the mechanism by which gold may induce nephropathy, the infrequency of proteinuria or nephrotic syndrome and the lack of correlation with dosage or blood and urinary levels of gold suggest that hypersensitivity is involved. Also, it is difficult to determine whether the nephrotic syndrome is due to membranous glomerulonephritis alone or with accompanying lesions, e.g., chronic tubulointerstitial nephritis, not uncommonly observed in rheumatoid arthritis.

Heroin

Nephrotic syndrome has been reported in heroin addicts. The renal histopathological changes are consistent with sclerosing glomerulonephritis or focal glomerular sclerosis. In a study of eight heroin addicts, Kilcoyne and colleagues

(1972) found focal membranoproliferative glomerulonephritis by LM and discrete areas of GBM thickening with granular electron-dense deposits on the subendothelial aspect of the GBM by EM. The deposits were found to be mostly IgM by IFM. Recently, Grishman and colleagues (1976) have reported details of glomerular morphology by LM, EM, and clinical manifestation in 23 heroin addicts who presented with nephrotic syndrome. In two-thirds of the cases, LM and EM findings were compatible with focal glomerular sclerosis. The clinical spectrum of progressive renal failure in 50% or more of these patients and persistent proteinuria in the remaining patients supports the diagnosis of focal glomerular sclerosis. Since there is no known etiology for focal glomerular sclerosis (and the existence of this entity is controversial), it becomes difficult to attribute the aforementioned renal lesions to heroin or the contaminants or otherwise. Furthermore, heroin addicts are prone to a variety of infections and the renal lesions observed could conceivably occur as a result of immunological mechanism(s) initiated by the infectious processes. Because the histopathological findings are consistent in both series and because of their resemblance to experimental aminonucleoside and *N,N'*-diacetylbenzidine-induced nephrosis, the heroin-associated nephropathy appears to have a toxic basis.

RENAL VEIN THROMBOSIS VERSUS MEMBRANOUS GLOMERULONEPHRITIS

Although the association between unilateral or bilateral renal vein thrombosis and membranous GN is an established fact, the cause and effect relationships between the two entities remain unsettled. There is a general consensus of opinion that renal vein thrombosis is the result and not the cause of membranous GN, although there is both favorable and unfavorable evidence for each notion. The evidence that contradicts the hypothesis that renal vein thrombosis is primary to membranous GN is as follows:

1. In a 3-month-old baby, unilateral renal vein thrombosis failed to demonstrate GBM changes characteristic of membranous glomerulonephritis.
2. Except during infancy, primary renal vein thrombosis is rare, probably because of the rapidity of blood flow. In a review of 228 cases of renal vein thrombosis, 40% of patients were babies. In infancy, renal vein thrombosis occurs especially as a complication of acute gastroenteritis and dehydration.
3. In adults, renal vein thrombosis may occur as a secondary and often terminal complication of renal disease. Thus, it may be found in glomerulonephritis and pyelonephritis, and is particularly liable to occur in renal amyloidosis. In an autopsy study of 249 cases, renal amyloidosis was found in 13 cases. Bilateral renal vein thrombosis was found in all but one case of amyloidosis.
4. A group of 30 patients with nephrotic syndrome due to membranous or proliferative glomerulonephritis was studied by inferior venacavagram and selec-

tive renal venogram by a group of investigators. Although a greater number of patients with renal vein thrombosis had membranous GN, no difference was found in the ultrastructure of glomeruli in the group with renal vein thrombosis and in the group without it. Margination of PMN was found in three patients. We have consistently found margination of neutrophil leukocytes in membranous glomeronephritis associated with unilateral or bilateral renal vein thrombosis (Figs. 7-5 and 7-7). This finding is considered by some as an important differential feature of idiopathic membranous GN and membranous GN associated with renal vein thrombosis. It should be remembered that margination of PMN is not a specific finding since it can be observed in any acute or chronic immune complex glomerulonephritis. Regardless of the cause and effect relationship, certain clinical features and laboratory tests appear to characterize renal vein thrombosis. Cade and associates (1977) have reported a constellation of findings in 28 patients with documented renal vein thrombosis. These findings, shown in Table 7-1, occurred with such frequency in these patients that the authors believe it virtually diagnostic of renal vein obstruction. These authors have also considered that these findings are sufficiently valuable to indicate renal venography and biopsy procedures. We believe that a combination of these clinical features and the

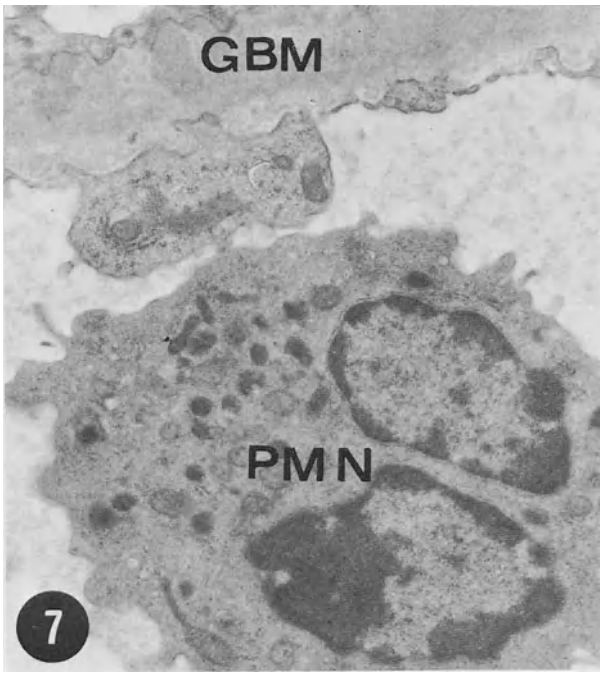


Fig. 7-7. Margination of neutrophil leukocyte (PMN) to the glomerular basement membrane (GBM), which reveals stage 3 membranous glomerulonephritis (UA + LC, ×12,000). From the first renal biopsy in a patient who developed thrombosis of inferior vena cava and both renal veins following severe injuries in the abdomen and back caused by an automobile accident.

Table 7-1
Frequency of Clinical and Laboratory Findings in 28 Adolescent and Adult Patients with Chronic Renal Vein Thrombosis

Finding	Number of patients	Number of patients observed
Edema	28	28
Nephrotic syndrome urine protein >3.5 g/24 hr	27	28
Greater than 2:1 variation in urinary protein loss (within a 2-week period)	14	20
Extrarenal thromboembolic disease	17	28
Pyuria (urine culture negative in 26 patients)	27	28
Hematuria	24	28
Reduced creatinine clearance	28	28
Interstitial fibrosis	26	28
Renal tubular dysfunction	25	28
Glycosuria	20	28
Renal tubular acidosis	16	28
Asymmetric kidneys	17	28
Hypertension	19	28
Flank pain	18	28
Increased fibrin degradation products	17	17

Source: From Cade *et al.* (1977), by the kind permission of Dr. Robert Cade, University of Florida, Gainesville, and the editor of the *American Journal of Medicine*.

laboratory tests (Table 7-1) along with the histological findings of interstitial fibrosis and margination of neutrophil leukocytes in the glomerular capillaries should suffice to denote a syndrome of chronic renal vein thrombosis.

5. Serial renal venography studies have shown development of renal vein thrombosis at a later date in patients with previously documented membranous glomerulonephritis.

6. Nephrotic syndrome occurs in animals only if, in addition to constriction of unilateral renal vein, the contralateral kidney is removed. The renal pathology in experimental nephrotic syndrome is not consistent with membranous glomerulonephritis.

7. The nephrotic syndrome in SLE is often found to be associated with renal vein thrombosis and membranous GN. Since SLE can produce membranous GN, this is further evidence in support of the concept that renal vein thrombosis is an effect rather than a cause of membranous glomerulonephritis.

8. In a patient with unilateral renal vein thrombosis, proteinuria and nephrotic syndrome disappeared following anticoagulant therapy. Recanalization of the affected renal vein was demonstrated but the pathology of the kidneys progressed.

9. Thromboembolism, especially pulmonary embolism, is often a presenting feature and heavy proteinuria or nephrotic syndrome is often discovered at that time.

The evidence that supports the hypothesis that renal vein thrombosis is primary to membranous glomerulonephritis is as follows:

1. Patients with unilateral renal vein thrombosis have demonstrated membranous GN in the contralateral kidney.
2. Proteinuria has been shown to be qualitatively and quantitatively identical bilaterally in unilateral renal vein thrombosis.
3. Complete disappearance of proteinuria and reversibility of renal morphology to normal following heparin treatment have been reported in a patient with documented bilateral renal vein thrombosis.
4. Cade *et al.* (1977) have found excessive interstitial fibrosis consistently in renal biopsies from chronic renal vein thrombosis. The interstitial fibrosis may be the sequelae of interstitial edema which is almost always observed in renal vein thrombosis (Fig. 6-37).

The bulk of evidence favors renal vein thrombosis as a complication secondary to membranous GN. The questions that arise in this regard concern how thrombosis occurs in the renal vein(s), and why one renal vein is affected more than the other. Neither question can be answered effectively. With respect to the first, GBM is thrombogenic; in membranous GN, there is excessive destruction of the GBM, and GBM can be eluted from the urine of patients with membranous GN. It may be conjectured that GBM products may be instrumental in the initiation of thrombosis. The hypercoagulable state demonstrated by the increased levels of factors V, VI, VIII, and X, fibrinogen, and platelets found along with renal vein thrombosis and membranous GN is a supportive finding. This abnormal coagulation state may explain thrombosis of the peripheral vein and the thromboembolic phenomenon in membranous GN. Normalization of the clotting factors during clinical remission, despite persistent GBM damage, is difficult to explain. If renal vein thrombosis is part of a hypercoagulable state, why does it then involve one renal vein rather than both? In another study, excessive urinary excretion and decreased serum level of antithrombin III were found in membranous glomerulonephritis and nephrotic syndrome. It has been suggested that the loss of this circulatory anticoagulant may be conducive to thrombotic tendency in membranous GN. It does not explain, however, why thrombosis will occur preferentially in the left renal vein.

Despite the weighty arguments against the proposition that renal vein thrombosis is the cause of membranous GN, this possibility, based on (1) clinical and circumstantial evidence and (2) histological differences, still exists. There are reports on individual patients' studies that support the idea that renal vein thrombosis is a causative factor.

After reviewing both supporting and opposing views, it is now clear that primary renal vein thrombosis, irrespective of etiology, can produce heavy proteinuria and nephrotic syndrome. The fact that proteinuria in most instances was unassociated with discernible glomerular damage and almost always disappeared, especially after anticoagulant treatment, further contradicts the view that renal vein thrombosis is a direct cause of membranous GN.

Whatever the circumstances, the relationship between renal vein thrombosis and membranous glomerulonephritis remains an enigma. Induction of membranous glomerulonephritis in experimental animals, as well as serial renal venography prior to initiation of the experiment and thereafter, may answer this ambiguous question.

Radiographic Manifestations of Renal Vein Thrombosis

A renal arteriogram is recommended by some radiologists as the first step in the radiological diagnosis of renal vein thrombosis. This allows evaluation of possible intrarenal pathology, such as renal neoplasm, and, during the venous phase, can demonstrate collateral drainage. The most definitive diagnositc modality is phlebography, including examination of the inferior vena cava and renal veins. It should be remembered, however, that selective renal venography could result in clot dislodgement.

With standard selective renal phlebography, the contrast material is injected retrograde into the renal vein. This method fills only the main renal vein and major redicles, so that small thrombi in the finer intrarenal veins, even back to the arcuate system, cannot be demonstrated. The technique involves injecting a very small amount of aqueous epinephrine into the respective renal artery in order to reduce renal blood flow during the retrograde venous injection.

NEPHROTIC SYNDROME ASSOCIATED WITH SICKLE CELL ANEMIA

The histopathological spectra encountered in nephrotic syndrome associated with sickle cell anemia are atypical mesangioproliferative (mesangiocapillary) glomerulonephritis, immune complex glomerulonephritis, and lipoid nephrosis.

Atypical mesangioproliferative GN is termed thus because it reveals all but mesangial deposits. The mesangial deposit along with deposits in the peripheral capillary loops characterize typical mesangioproliferative GN. The LM findings reveal slight mesangial hypercellularity and irregularly thickened GBM. EM study has demonstrated mesangialization of the peripheral capillary loops, i.e., two layers of basement membranes, the outer thicker than the inner and interposition between the two layers of basement membranes, cells resembling endothelial cells, scattered granules approximately 250 Å (1 Å = 0.1 mμ) in diameter, and the absence of electron-dense deposits (Figs. 7-8a and 7-8b).

The characteristic EM finding ot immune complex GN has been variously described as subepithelial electron-dense deposits, humps, or even typical "humps." In view of the latter finding, a diagnosis of poststreptococcal GN has often been made, a diagnosis supported in most instances by a history of sore throat, positive throat culture for beta hemolytic streptococcus, elevated ASO titers, and granular fluorescence for IgG and C3.

There are no data, however, to suggest that the incidence of poststreptococcal glomerulonephritis is higher in sickle cell anemia (SS disease) than in the general population. Rather, the high incidence of pneumococcal infection in SS disease may subject these individuals to immune complex GN and even atypical mesangiocapillary GN as a result of pneumococcemia.

A variety of histopathological changes in the kidney in sickle cell anemia raises a question with regard to the relationship between sickle cell anemia and various renal lesions. There is no direct evidence thus far to indicate a direct cause and effect relationship between renal disease and sickle cell anemia. Some authors have considered this association to be fortuitous; experimental studies do not support any causal relationship either. It was thought that excessive amounts of iron released as a result of hemolysis and their entrapment by the glomerular mesangium might have caused atypical mesangioproliferative glomerulonephritis. Intravenous injection of saccharated iron oxide into rabbit caused nephrotic syndrome and resulted in heavy accumulation of iron particles in the mesangium but there was no demonstrable change in the GBM. It was thought that nephrotic syndrome in sickle cell anemia could be due to iron overload. This is most unlikely in the human since glomeruli from SS disease have demonstrated slight or no Prussian blue-stainable iron. We have found no iron particles in the glomeruli studied by EM in renal biopsies from two patients with SS disease. The clinical picture and EM study of renal biopsy have been elaborated in the following case:

E.B., a 17-year-old black male with sickle cell disease, had numerous admissions to the Oklahoma Children's Memorial Hospital for a variety of complaints relative to SS disease. At age 7 a urinalysis showed trace protein, 0 to 2 RBC, and 0 to 1 WBC. At age 8 he noticed swelling of both lower extremities. Two weeks prior to swelling he had had a cold which lasted for 2 days, but he denied bloody urine. His older brother had SS disease and developed similar swelling. A renal biopsy study from the brother revealed atypical (type III) mesangioproliferative glomerulonephritis (Figs. 7-8a and 7-8b). Laboratory studies—Urinalysis: 2+ protein, rare hyaline, and RBC cast; serum urea nitrogen 12 mg/100 ml; ASO titer 625 Todd units; throat culture normal flora. A percutaneous renal biopsy was performed and the renal tissue was studied by LM and EM, both of which revealed acute diffuse proliferative glomerulonephritis (Fig. 7-9). EM revealed aggregates of platelets in the glomerular capillaries and no electron-dense deposits. Urinalysis during subsequent admissions for the following 8 years showed persistent 2+ to 3+ proteinuria with slight or no sediment.

Since the two brothers with SS disease had inconsistent renal pathology, two plausible explanations can be offered: (1) A variety of infections may cause diffuse proliferative glomerulonephritis and atypical mesangioproliferative glo-

merulonephritis, or (2) an unknown factor in SS disease causes a variety of lesions in the kidney.

Strom *et al.* (1972) did not find any iron deposit on Prussian blue stain and electron microscopy of the renal tissue in a patient with sickle cell anemia. Their patients had demonstrable obstruction of the right renal vein. Two consecutive bilateral renal biopsies were done; the first biopsy showed marked interstitial edema, large-sized glomeruli, and fusion of the epithelial foot processes; the second biopsy revealed glomerular changes consistent with stage 1 membranous GN. The authors attributed the progressive pathological changes in the kidney to renal vein thrombosis.

There seems to be some association between lipoid nephrosis and SS disease. Intravenous injection of iron has been shown to produce damage in the glomerular

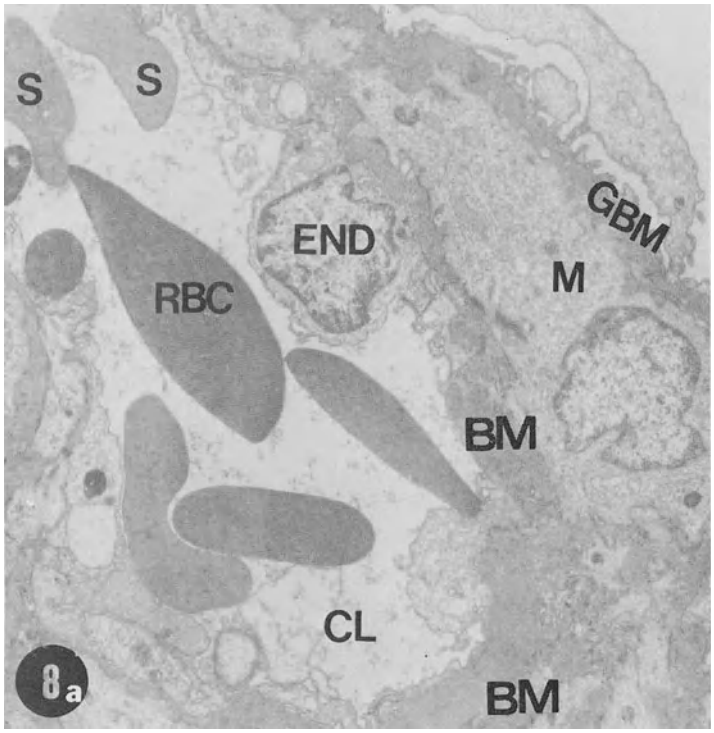


Fig. 7-8. (a) Mesangialization of the peripheral capillary loop. Formation of a mesangium (M) between the glomerular basement membrane (GBM) and basement membrane-like material (BM) is seen. Endothelial cell (END) is separated from the mesangial cell by the BM. Within the lumen of the capillary (CL), normal red blood cells (RBC) and sickled cells (S) are shown (UA + LC, ×5000). (b) Mesangialization is a conspicuous feature in this micrograph. Many mesangiumlike cells (M) and basement membrane-like (BM) materials intervene between glomerular basement membrane (GBM) and endothelial cell (END). Small discrete electron-dense deposits (arrowheads) in the subendothelial location are shown. Normal red blood cells (RBC) in the lumen of the original capillary (CL) and mesangialized capillaries are shown (UA + LC, ×5000).

epithelial cell foot processes. Even though iron accumulation does not occur in human glomeruli, iron may induce fusion of epithelial cell foot processes during filtration through the glomeruli.

NEPHROTIC SYNDROME IN ACQUIRED SYPHILIS

There are several reports concerning glomerulonephritis and nephrotic syndrome in acquired syphilis. Review of reported clinical and renal histopathological studies reveals consistent and characteristic patterns of syphilitic glomerulonephritis, including (1) occurrence in secondary syphilis; (2) nephrotic syndrome as the most common presentation; (3) normal blood pressure; (4) normal renal function; (5) normal serum C3 level; (6) normal histology or mild proliferative glomerulonephritis by LM study (Fig. 7-10); (7) immune complex type of glomerulonephritis, spiky GBM, and discrete electron-dense deposits by EM. The deposits are strictly epithelial and appear as atypical "humps" (see Figs. 6-5 and

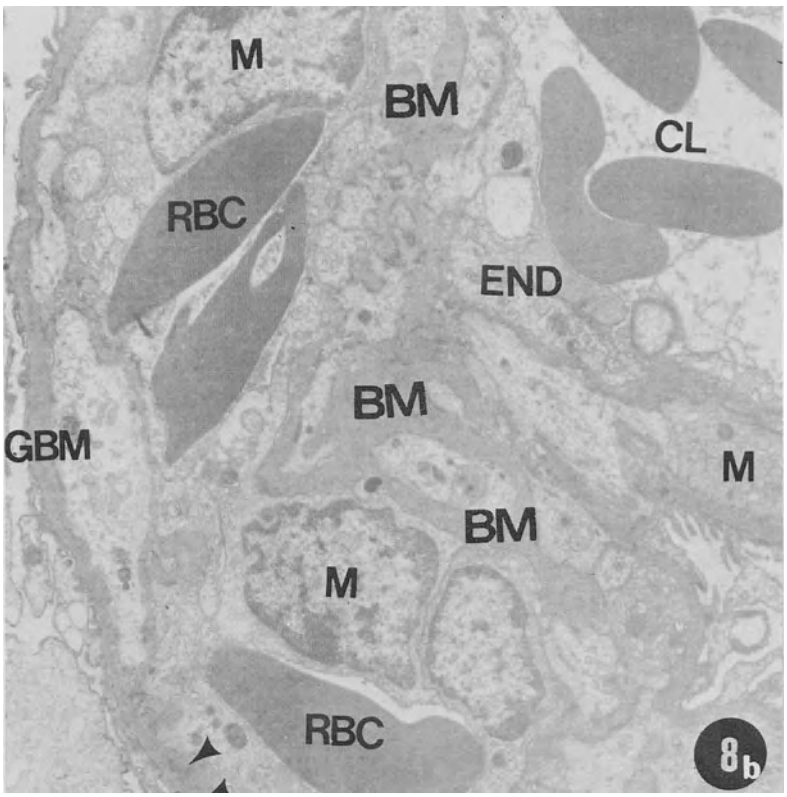


Fig. 7-8. *(Continued)*

7-11) due to the incomplete or absent limiting membranes of electron-dense deposits. The fusion of foot processes is more extensive and uniform than that observed in glomerulonephritis associated with typical "humps." (8) Granular fluorescence of mainly IgG or IgG and C3 in the peripheral capillary loops as well as in the mesangium of the glomeruli by IFM; (9) positive serological tests for syphilis; (10) good response of the nephrotic syndrome to penicillin therapy.

A brief summary of the patient studied by Braunstein *et al.* (1970) is cited here by the kind permission of Dr. G. D. Braunstein, University of California at Los Angeles, to demonstrate the clinical response of the nephrotic syndrome to large doses of penicillin.

An 18-year-old black woman was admitted to the Peter Bent Brigham Hospital on February 28, 1969 with an 8-day history of painless swelling of the eyes and legs as well as heavy proteinuria. Past history revealed excellent health except for two episodes of

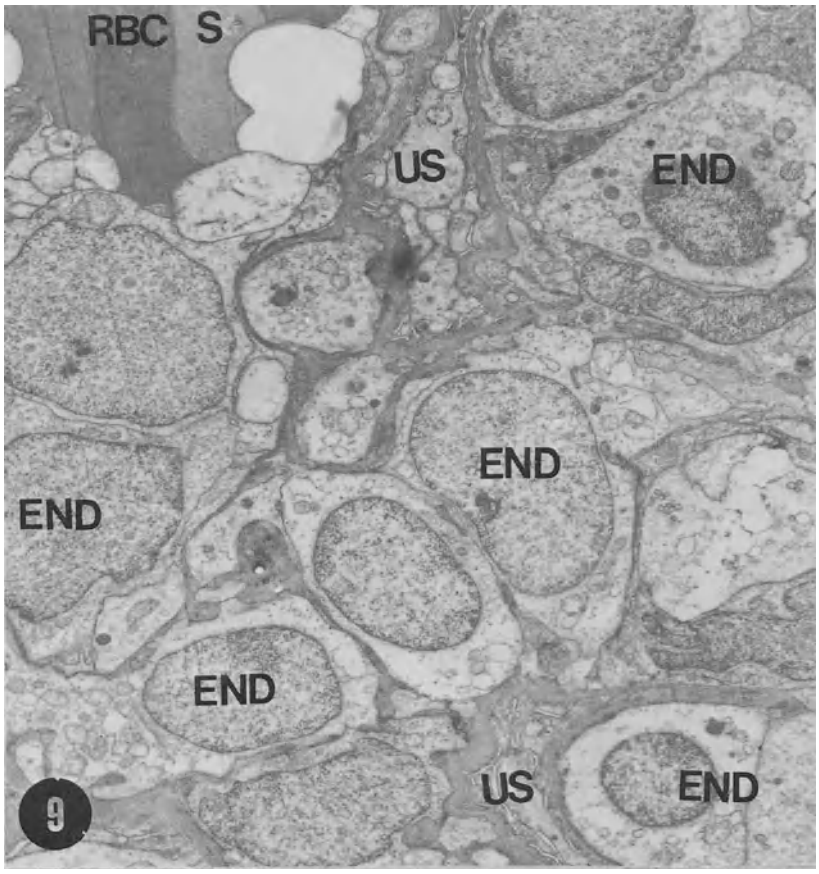


Fig. 7-9. Marked proliferation of endothelial cells (END) which has occluded the lumina of glomerular capillaries and compromised the urinary space (US). Normal erythrocytes (RBC) and sickled erythrocytes (S) are seen within the lumen of a capillary (UA + LC, ×5500).

gonorrhea which were treated with penicillin. Six months prior to admission, the patient had been evaluated in a prenatal clinic when she was 4 months pregnant. She weighed 149.5 lb, had a blood pressure of 110/60 mm Hg, normal urine, and negative serum Hinton test. Her prenatal course was uneventful, but at 33 weeks' gestation, following premature rupture of the membranes, she was delivered of a 3 lb, 15 oz female baby. Culture of the infant's eyes grew *Neisseria gonorrhoeae,* and the infant, but not the mother, was treated with systemic penicillin and ophthalmic erythromycin.

Five weeks later, the patient was readmitted with a history of sore throat, nasal congestion, and a dry, nonproductive cough of 2 weeks' duration. This was followed by a macular–papular erythematous symmetrical eruption over the flexor surface of her arms, face, neck, chest, back, abdomen, and legs.

The patient's contact had been seen at another hospital in December 1968, because of a nonhealing penile ulceration in which *Treponema pallidum* was demonstrated, and appropriate therapy was given.

Physical examination revealed the patient to be moderately obese and edematous, but in no acute distress. A fading erythematous macular rash was present on the face and the flexor surfaces of the arms. There was marked periorbital edema and puffiness in the malar area symmetrically. The nasal septum was intact. A grade 2/6 early short systolic murmur was present along the left sternal border. Pelvic examination revealed several purulent papular lesions on the labia and normal menstrual flow. There was 3+/4+

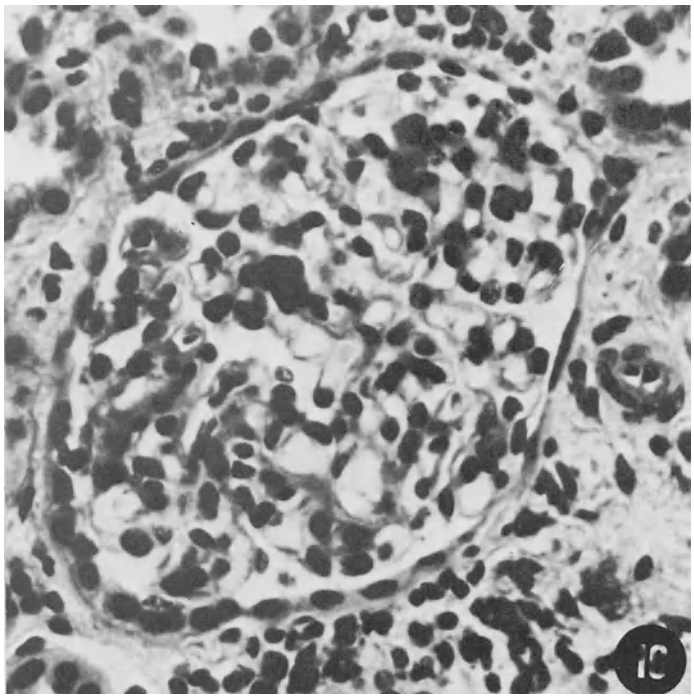


Fig. 7-10. This glomerulus reveals slight mesangial hypercellularity only. The thickness of the peripheral capillary loops appears to be within normal limits. Reduced population of tubules and slight interstitial fibrosis are observed (H & E, ×200). From the renal biopsy of a patient with nephrotic syndrome ostensibly due to secondary syphilis.

pretibial, pedal, and hand-pitting edema. There was no lymph node enlargement. The remainder of the physical examination was within normal limits.

Urinalysis revealed hazy yellow color, pH of 6.5, specific gravity of 1.038, and heavy proteinuria. No sugar or acetone was present. Spun urine sediment revealed five to six granular casts, *Trichomonas vaginalis,* many epithelial cells, free fat, oval fat bodies, and 10 to 15 WBC/HPF. The urine contained 10 g protein/24 hr on admission. No red blood cells or red blood cell casts were observed.

The erythrocyte sedimentation rate (Wintrobe) was 34 mm. The hematocrit was 40% and the white blood cell count 6450/mm^3, with 72% segmented neutrophils, 23% lympho-

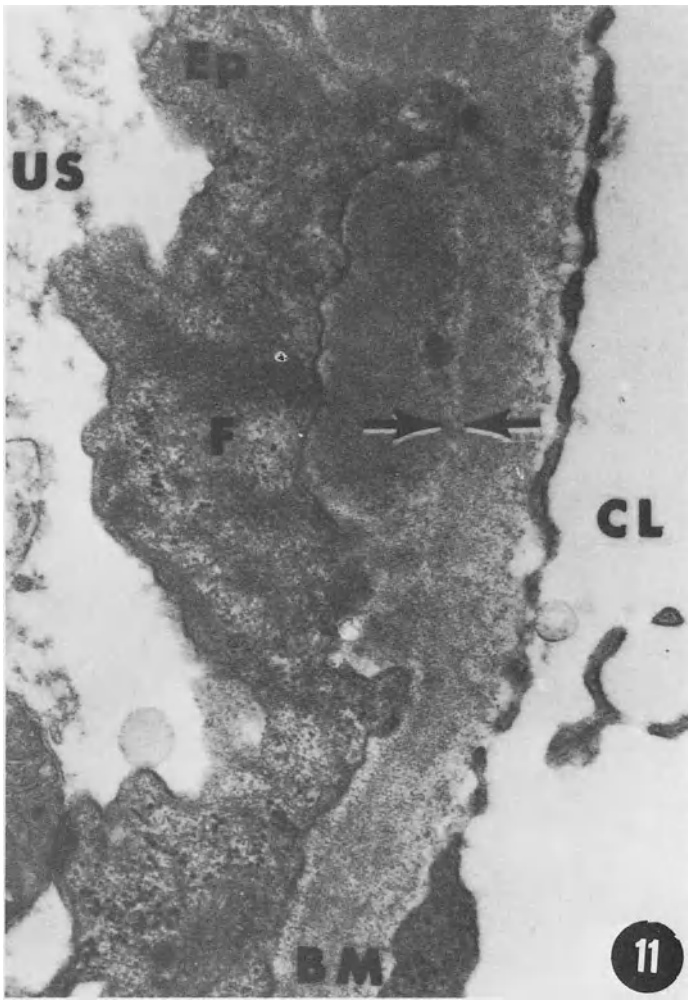


Fig. 7-11. The electron-dense deposits along with the widespread fusion of foot processes (F) observed here and in Fig. 6-5 appear to be consistent with stage 1 membranous glomerulonephritis. Separation between glomerular basement membrane (BM) and electron-dense deposit (opposing arrows), lumen of the capillary (CL), and urinary space (US) can be seen (UA + LC, ×22,000). From the same patient as in Fig. 7-10.

cytes, and 5% monocytes. An eosinophil count was 31/mm^3. Hemoglobin electrophoresis revealed an SA pattern.

The BUN, creatinine, electrolytes, fasting, and 2-hr postprandial blood sugar levels, serum enzymes, and other chemistries were normal. ASO titer was normal. Protein electrophoresis revealed a total protein of 5.6 g/100 ml with an albumin of 1.3 g/100 ml, alpha$_1$ globulin 0.6 g/100 ml, alpha$_2$ globulin 1.2 g/100 ml, beta globulin 1.2 g/100 ml, and gamma globulin 1.3 g/100 ml. Cryoglobulins were absent. Triglycerides were 610 mg/100 ml and cholesterol 325 mg/100 ml. Rheumatoid factor, LE cell, and ANA were negative. She had normal serum C3 and normal whole hemolytic complement in the serum.

Repeated blood, urine, and cervical cultures were sterile. The Hinton test was strongly positive up to a dilution of 1:128. The Reiter protein complement fixation test (RPCF) was positive. Fluorescent treponemat antigen absorption test (FTA-abs) was 1+/4+ reaction initially, and when repeated 2 weeks later, was 4+/4+.

The chest roentgenogram was normal. An intravenous pyelogram was essentially within normal limits and revealed equal and prompt dye excretion bilaterally. The electrocardiogram was normal.

She was placed at bed rest, on a high-protein, 1.0-g sodium chloride diet, and a 24-hr fluid restriction to 1200 ml daily. On the ninth hospital day, an open left renal biopsy was performed under spinal anesthesia and 2 days later treatment was begun with procaine penicillin. The clinical course is shown in Fig. 7-12. Approximately 8 hr after the institution of therapy, a Herxheimer reaction, manifested by fever, malaise, and joint pains, was witnessed. The patient was discharged on the 15th hospital day and continued to take her penicillin as an outpatient with regular clinic follow-up. By the ninth day after discharge,

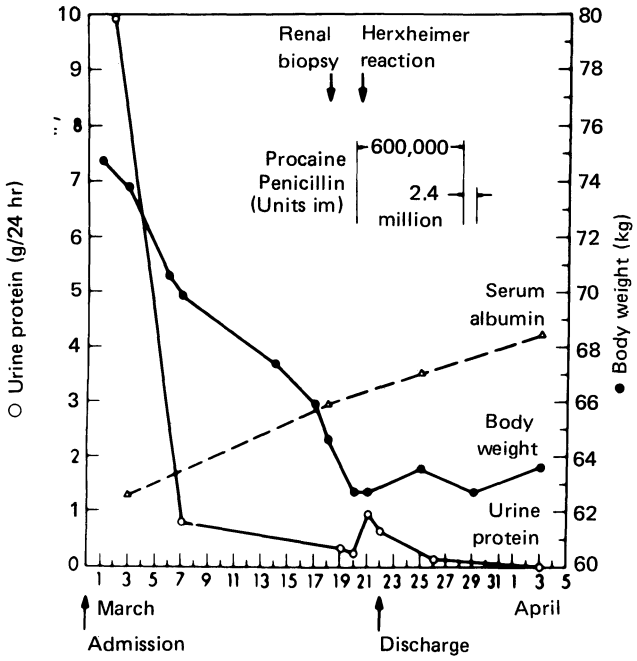


Fig. 7-12. Clinical course. Note rapid drop in proteinuria and body weight and increase in serum albumin even before penicillin was started. The proteinuria disappeared after penicillin administration. From the same patient as in Fig. 7-10.

she was free of edema, her body weight had stabilized, the serum albumin was normal, and there was no proteinuria.

Several questions arise with regard to syphilitic glomerulonephritis: (1) Is it a distinct entity caused by treponemal antigen? (2) How does it produce nephrotic syndrome? (3) How does nephrotic syndrome reverse following penicillin therapy? The favorable and unfavorable features of the specificity of syphilitic GN are listed in Table 7-2.

The evidence for and against *T. pallidum*-induced glomerulonephritis is balanced almost equally. In any case, the overwhelming clinical evidence, especially prompt disappearance of nephrotic syndrome following penicillin administration, establishes the fact that nephrotic syndrome is caused by secondary syphilis.

It needs to be determined whether the syphilitic glomerulonephritis is the result of a single episode of syphilitic infection similar to one-shot serum sickness nephritis or results from recurrent episodes of subclinical syphilitic infection similar to two-shot serum sickness nephritis. The EM findings of atypical "humps," spiky GBM in some capillaries, and extensive fusion of foot processes in most glomerular capillaries, suggestive of membranous GN, favor the latter mechanism. This is supported further by normal serum C3. In addition, acute immune complex glomerulonephritis, such as poststreptococcal GN and glomerulonephritis of bacterial endocarditis, is almost invariably accompanied by moderate to severe exudation of neutrophil leukocytes within the glomeruli, which is not found in syphilitic GN.

Table 7-2
Features of the Specificity of Acquired Syphilitic Glomerulonephritis

Pro	Con
Clinical manifestations only during secondary stage of syphilis	Absence of florid proliferative and exudative changes in the glomeruli by LM
Consistency in clinical presentations and histopathological findings in all the reported series	Lack of unequivocal evidence of the presence of *Treponema pallidum* in the immune complex
Absence of past history of renal disease, hypertension, diabetes mellitus, etc., in all reported series	Widespread fusion of foot processes being unusual in acute immune complex glomerulonephritis
Renal biopsy elution studies demonstrate the antibody component of the complexes specifically directed against treponemal antigen	Lack of long-term follow-up studies to document permanent disappearance of proteinuria following penicillin therapy
Dramatic and complete response of the nephrotic syndrome to large dose of penicillin in all the reported series	No serial renal biopsy study to document reversibility of renal histopathology to normal after penicillin therapy

How does syphilitic glomerulonephritis produce nephrotic syndrome? It is difficult to relate the histopathological changes to heavy proteinuria in any of the patients studied. The GBM appearance does not warrant heavy proteinuria. On the other hand, it seems reasonable to attribute proteinuria to extensive foot process fusion. It is not known whether syphilis can produce foot process change similar to that observed in lipoid nephrosis.

The most difficult question to answer concerns how penicillin reverses nephrotic syndrome. The destruction of spirochetes in the blood and tissue by penicillin in some unknown way may cause the disappearance of protein leak by the glomeruli. Clinical follow-up of some of these patients along with determination(s) of 24-hr proteinuria and serial renal biopsy studies should substantiate the mechanism of syphilitic glomerulonephritis.

NEPHROTIC SYNDROME IN CONGENITAL SYPHILIS

The renal histopathological changes in nephrotic syndrome due to congenital syphilis have been reported to consist of mesangial cell proliferation, increased mesangial matrix, and irregular thickening of basement membranes or epimembranous glomerulopathy. A group of investigators from Capetown, South Africa have demonstrated immune complex glomerulonephritis in four infants with congenital syphilis who manifested nephrotic syndrome. Although a variety of discrete electron-dense deposits had been observed, some of the glomerular capillaries exhibited GBM changes and the extramembranous deposits characteristic of idiopathic membranous glomerulonephritis (see Figs. 7-14 and 7-15; Fig. 7-13 is the LM study). By IFM, coarse granular fluorescence for IgG and C3 was found in three of four patients. Additional supportive features for nephrotic syndrome due to congenital syphilis are (1) elevated serum IgM level, (2) prompt clinical response of nephrotic syndrome to penicillin therapy, (3) progression of histopathological changes despite clinical remission, and (4) the worsening of the histopathological changes attributable to excess antigenic stimuli by penicillin.

The pathogenesis of glomerulonephritis in congenital syphilis is unclear. It is possible that spirochetemia occurring *in utero* as a result of transplacental passage of the organism may incite antibody production and form excessive amounts of antigen–antibody complex, a mechanism that is analogous to immune complex GN in secondary syphilis. If this were the mechanism and the intrauterine fetus played the major role in immune complex formation, the antibody would have been largely and consistently IgM. In contrast, IgG, which appears to be derived from the mother, was the predominant antibody found by IFM study. Therefore, it appears that the mother forms immune complexes which pass through the placenta and affect the fetal kidney. However, this hypothesis cannot explain the freedom of the mother from immune complex glomerulonephritis.

NEPHROTIC SYNDROME CAUSED BY MALARIAL GLOMERULONEPHRITIS

Wing and co-workers (1971) studied nephrotic syndrome in 156 patients at Millago Hospital, Kampala, Uganda. Since malaria is hyperendemic in and around Kampala, this study was undertaken to determine the incidence of malaria as a direct cause of nephrotic syndrome. Approximately 80% of patients were studied for malarial parasitemia by the multiple slide technique, malaria antibody titer was measured in about 70% of patients, and renal biopsies were studied in 68% of the patients. ASO titers and serum C3 were measured in some of these cases.

Based on the results of the studies, the authors have suggested that in Uganda and similar geographical areas, nephrotic syndrome associated with mild

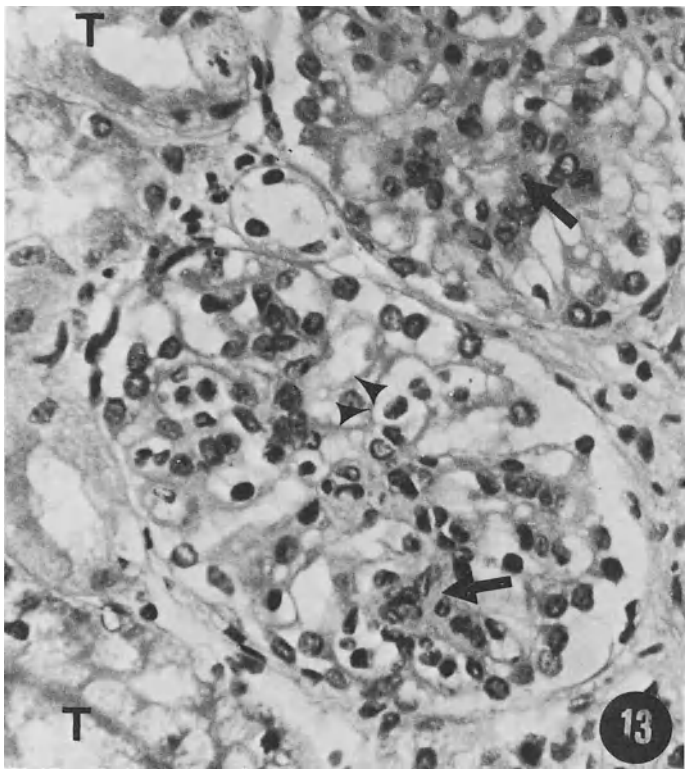


Fig. 7-13. The two glomeruli reveal proliferative changes involving mainly the mesangial areas. Increase in mesangial matrix (arrows), mild thickening of peripheral capillary loops (arrowheads), and synechiae are observed. Tubules (T) show swelling of the epithelial cells (H & E, ×200). From the renal biopsy of a 2-month-old cape-colored male baby. He had positive serology for syphilis (WR, VDRL, and FTA), 260 to 990 mg protein/100 ml urine, serum albumin 1.0 g/100 ml, serum creatinine 0.4 mg/100 ml, ASO titer 125 Todd units, and serum whole complement 182 mg/100 ml.

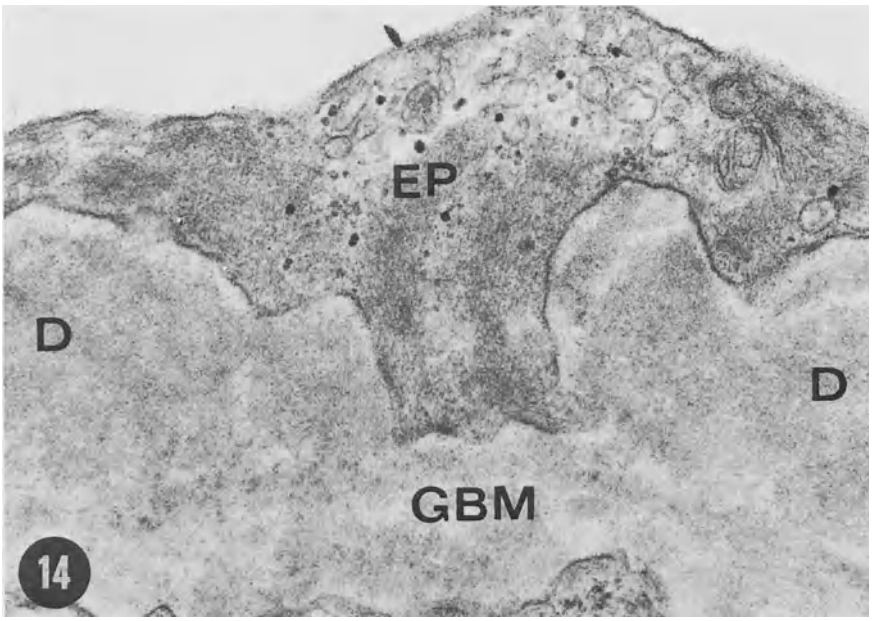


Fig. 7-14. Electron microscopy of the renal biopsy from the same baby as in Fig. 7-13 demonstrates marked thickening and spiking of the glomerular basement membrane (GBM) and fine granular electron-dense deposits (D) within the GBM. Fusion of epithelial cell and foot process (EP) is shown (UA + LC, ×15,000).

focal and often segmental glomerular lesions is due to quartan malaria. This is more common in children than in adults. Nephrotic syndrome in patients showing generalized proliferative lesions on renal biopsies may be due to quartan malaria, streptococcal infection, or otherwise. Immunofluorescence microscopy study did not aid much in distinguishing between glomerulonephritis due to malaria and that due to streptococcal infection. Therefore, the authors have concluded that epidemiological, parasitological, and immunological studies provide strong evidence that nephrotic syndrome in the majority of children and in many adults is associated with quartan malaria. None of the histopathological features, however, can be considered specific for malaria, and the evidence for malarial GN is only circumstantial.

The histological patterns of malarial glomerulonephritis among 40 children from Nigeria, described by Williams (1976),* included proliferative, membranoproliferative (mesangioproliferative), minimal change, and membranous glomerulonephritides.

> Ultrastructural studies of renal biopsies revealed the presence of deposits in the mesangial, epimembranous, intramembranous and subendothelial areas. Mesangialization of the peripheral capillary loops had been observed. This syndrome is associated

* Excerpted through the kind permission of A. O. Williams, M.D., University College Hospital, Ibadan, Nigeria.

with non-selective proteinuria and response to corticosteroid therapy is not good. The renal lesions progressed, with patients subsequently dying from secondary hypertension and renal failure.

These cases of glomerulonephritis have been attributed to infection with quartan malaria because quartan malaria parasitemia occurs in 80% of Nigerian children with nephrotic syndrome, and reinfection with malaria predisposes to exacerbations of symptoms and rapid progression of renal damage. Although immunological studies demonstrated malarial antigen, yet the histological patterns, progression of the disease processes, and poor or no response to therapy are not different from glomerulonephritis of non-malarial origin.

Therefore, although the circumstantial evidence of malarial parasitemia and the presence of malarial antigen in the immune complexes supports glomerulonephritis of malarial origin, there is no solid evidence yet for this conclusion.

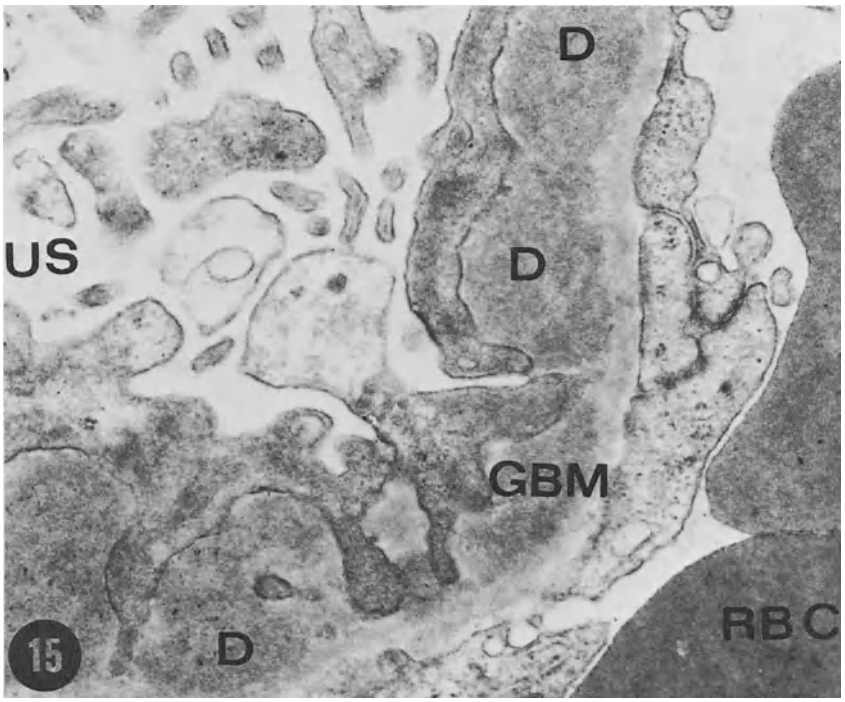


Fig. 7-15. This electron micrograph shows two discrete layers of glomerular basement membrane (GBM) similar to those shown in Figs. 7-5 and 7-6. In the outer spiky layer granular electron-dense deposits (D) are discerned. These GBM changes are somewhat consistent with stage 2 membranous glomerulonephritis. Erythrocytes (RBC) can be seen within the lumen of the glomerular capillary. Urinary space (US) is shown (UA + LC, ×10,000). From the renal biopsy of a 4-week-old male baby. Renal biopsy was done after 10 days of administration of 3 mU of penicillin. All tests for syphilis were positive. He had 350 to 420 mg protein/100 ml urine, serum albumin 1.3 g/100 ml, serum creatinine 0.4 mg/100 ml, ASO titer 125 Todd units, and very low serum whole complement.

NEPHROTIC SYNDROME ASSOCIATED WITH NEOPLASTIC DISEASES

There is increasing evidence of nephrotic syndrome associated with a variety of malignant tumors, including solid tumors, lymphoma, and leukemia. With the exception of Hodgkin's disease, the tumor-related nephrotic syndrome appears to be due to membranous glomerulonephritis. In most cases of Hodgkin's disease accompanied by nephrotic syndrome, the renal histology is consistent with minimal lesion disease or lipid nephrosis. Cameron and Ogg (1974) reported a review of the world literature relative to the association of membranous GN with various malignant tumors. The results are summarized in Table 7-3.

A great deal of the literature tends to support a relationship between malignant tumors and glomerular diseases. Although the causal relationship is not yet established, the evidence indicates that the glomerular diseases are secondary to malignant tumors. An extensive review of tumor-related glomerular diseases has been reported by Eagen and Lewis (1977).

The unique features of tumor-related glomerular diseases are as follows:

1. They are more common in lymphoma than carcinoma.
2. That nephrotic syndrome may be a prodoma of malignant tumor is supported by the collective studies of Lee and associates (1966). These authors have emphasized that nephrotic patients 40 years or over should be carefully and repeatedly screened for cancer.
3. Membranous glomerulonephritis is the most common histopathological lesion observed.
4. There are a large number of reports which indicate that foot process

Table 7-3
Sites and Types of Neoplasia Associated with Membranous Nephropathy

Neoplasm	Number of patients reported
Carcinoma of bronchus	10
Carcinoma of colon or rectum	4
Hodgkin's disease	4
Carcinoma of mouth or pharynx	2
Carcinoma of breast	1
Carcinoma of skin	1
Carcinoma of ovary	1
Carcinoma, origin unknown	1
Wilm's tumor	1
Chronic lymphatic leukemia	1

Source: From Cameron and Ogg (1974), reproduced through the kind permission of J. Stewart Cameron, M.D., and colleagues, Guy's Hospital, London, England, and the editor of the *British Medical Journal.*

fusion disease or lipoid nephrosis is the most common cause of nephrotic syndrome in Hodgkin's disease. The less common causes of nephrotic syndrome include membranous glomerulonephritis and crescentic glomerulonephritis. The relationship between Hodgkin's disease and nephrotic syndrome is strengthened by the fact that nephrotic syndrome often relapses with reactivation of Hodgkin's disease.

5. Lymphosarcoma and reticulum cell sarcoma have been associated with glomerular lesions, including foot process fusion disease and membranoproliferative GN.

6. Tumor-associated antigen has been suggested as the stimulus for antibody production and antigen–antibody complex formation. The other antigens which may be operative in antigen–antibody complex formation are reexpressed fetal antigens, viral antigens, and autologous nontumor antigens.

7. The immunoglobulin elute from the kidneys of a patient with bronchogenic carcinoma and membranous glomerulonephritis cross-reacted with a preparation of antigens from the patient's tumor, but not from his normal lung tissue. Immunoglobulin with identical antigenic specificity was detected in the patient's serum. This finding supports the concept of glomerulonephritis mediated by tumor antigen–antibody complexes.

8. Immunofluorescence microscopy reveals granular fluorescence for IgG, C3 and C4, and IgM which fulfills the criteria of immune complex glomerulonephritis.

9. EM study has demonstrated membranous glomerulonephritis or lipoid nephrosis which are indistinguishable from the idiopathic types.

10. Removal of the solid tumor or its destruction by chemotherapy or radiotherapy is followed by amelioration of proteinuria and nephrotic syndrome in some instances.

11. Treatment of lymphoma almost uniformly causes remission of the nephrotic syndrome, especiallly when the nephrotic syndrome is due to lipoid nephrosis. Remission is less certain when membranous glomerulonephritis is the cause of nephrotic syndrome.

12. The clinical course of nephrotic syndrome associated with neoplasm is poor. In a study described by Eagen and Lewis (1977), 77% died within a relatively short time, the median survival being 12 months. About 20% survived for 1 year or longer. All these patients had progressive carcinomatosis as a major contributing factor to their deaths.

13. The overall review suggests that onset of nephrotic syndrome at 40 or afterwards heralds a possibility of a malignant tumor. The suspicion is important because treatment of the tumor may ameliorate the nephrotic syndrome.

CONGENITAL NEPHROTIC SYNDROME

SYNONYMS. Infantile nephrotic syndrome, infantile microcystic disease, congenital nephrotic syndrome of the Finnish type.

An increasing number of cases of nephrotic syndrome have been reported in newborns or infants since the mid-1940s. The nephrotic syndrome in this age group, unlike nephrotic syndrome in early childhood, is often familial. Although the term congenital nephrotic syndrome in general has been ascribed to nephrotic syndrome of early childhood, a type of severe nephrotic syndrome in the newborn can be recognized and separated as a distinct entity, aptly called congenital nephrotic syndrome. It is prevalent in Finland, but is not rare in other countries. Because of the known pattern of inheritance and variably fatal outcome of the disease, separation of the congenital nephrotic syndrome of the Finnish type from other nephrotic syndromes of the newborn and infants is of practical importance. In this regard, a precise classification of the nephrotic syndrome, including characteristic clinical features and renal histopathology in the first year of life, is described by Kaplan and colleagues (1974) and shown in Table 7-4.

The mode of inheritance of congenital nephrotic syndrome has been found to be autosomal recessive. There is a high incidence of toxemia of pregnancy, premature delivery, and large placenta. A patient with congenital nephrotic syndrome has been studied in depth, and is now described (from Mandal *et al.*, 1977*b*, by the kind permission of the editor of *Human Pathology*):

A 1-month-old white male baby born prematurely and with a large placenta was admitted to Oklahoma Children's Memorial Hospital five times in a period of 3½ months. The first admission was on June 5, 1975, for repair of bilateral inguinal hernia. Physical examination revealed 2+ to 3+ pitting edema in the extremities. The laboratory studies on initial admission and subsequent admissions are shown in Table 7-5. Subsequent admissions were for urinary tract infection, septicemia, and ascites. An open renal biopsy was done during the first admission and the specimen was studied by light, electron, and immunofluorescence microscopy.

Light microscopy. Most of the glomeruli had smaller tufts, giving the generalized appearance of enlarged Bowman's spaces (Figs. 7-16 and 7-17). The glomerular tufts appeared to consist of clusters of nuclei, most of which were located on the outer aspect of the capillaries. The capillary loops were barely recognizable. The majority of the tubules revealed a variable degree of vacuolated changes, some of which were converted into large clear spaces.

Electron microscopy. Three glomeruli were studied by EM. One glomerulus revealed a cluster of collapsed capillaries. These capillaries exhibited irregular thickening on the endothelial side of the basement membranes and linear density within the basement membranes. In the other two glomeruli, generalized and complete fusion of epithelial foot processes and thin GBM were observed (Figs. 7-18 and 7-19). Proximal tubular cells showed only cystic changes. These cysts caused disruption and disappearance of the cellular constituents. Despite the cystic changes, tight junctions between cells and the microvilli were intact in most tubular cells (Fig. 7-20). The basement membrane was thin in some tubules and irregularly thickened in others. The distal convoluted tubules, Henle's loops, and collecting tubules were unaffected.

Of four different large and small arterioles studied, one was normal, and three revealed cystic spaces in the endothelial and smooth muscle cells, in the basement membrane between the endothelial cells and smooth muscle cells, and in the basement membrane between individual smooth muscle cells (Fig. 7-21). Excessive amounts of elastic tissue were found between smooth muscle cells and around the periphery of arterioles. Masses of fibrin were found within the capillary and venous lumina and in the interstitium (Fig. 7-22).

Table 7-4
Classification of the Nephrotic Syndrome in the First Year of Life

Classification	Etiology	Age at onset	Noteworthy features	Pathology
Infantile microcystic disease (congenital nephrotic syndrome of the Finnish type)	Autosomal recessive	Birth to 3 mo	Toxemia of pregnancy, prematurity, placentomegaly. Nonresponsive to corticosteroid therapy	Dilated proximal renal tubules
Minimal lesion nephrotic syndrome	Usually idiopathic	6 mo	Usually responds to corticosteroid therapy	Fusion of foot processes on electron microscopy
Focal glomerular sclerosis	Idiopathic	6 mo	Microhematuria and corticosteroid resistance. Progressive renal failure may be seen	Focal segmental or diffuse glomerular sclerosis
Omniglomerular diffuse mesangial sclerosis	Idiopathic, possible familial	6 mo	Progressive development of renal failure	Omniglomerular diffuse mesangial sclerosis, interstitial fibrosis
Epimembranous glomerulopathy	Congenital syphilis	6 mo	Clinical features of congenital syphilis	Epimembranous deposits; epimembranous IgG and fibrin, or IgG, IgA, IgM, and C3 deposition
Conditions which may rarely be associated with the nephrotic syndrome		<2 yr	Genital anomalies	Glomerular sclerosis and interstitial fibrosis
		<2 yr	Nephroblastoma	
	Familial	1 case at birth usually >3–4 yr	Nail–patella syndrome	Focal glomerular sclerosis; by electron microscopy, lucent areas and collections of collagen fibrils in glomerular basement membrane
	Mercury intoxication	<2 yr	May have other clinical signs of mercury poisoning	

Source: From Kaplan *et al.* (1974), by the kind permission of the authors; The Montreal Children's Hospital, Montreal, Canada; and the editor of the *Journal of Pediatrics*.

Immunofluorescence microscopy. Except for scattered deposits of C3 in places other than basement membranes of glomeruli or tubules, all stained sections were negative.

Comments. Studies of kidney tissue from a 1-month-old premature baby with the nephrotic syndrome revealed cystic changes in the proximal tubules, multiple glomerular lesions, and remarkable arteriolar lesions. The glomerular findings were consistent with lipoid nephrosis in some glomeruli, but with the anti-GBM antibody type of glomerulonephritis or mesangial sclerosis in other glomeruli. Immunofluorescence microscopy has offered no convincing evidence of an immunopathogenic mechanism to account for the renal lesions.

In other studies of the congenital nephrotic syndrome, thin basement membranes and fusion of epithelial foot processes have been consistently observed. Several investigators have reported endothelial cellular hyperplasia and excessive basement membrane-like material in the mesangium. Habib and Bois (see Mandal *et al.*, 1977*b*) found diffuse mesangial sclerosis, similar to the findings in one glomerulus in our patient.

The negative immunofluorescence finding in the present study is consistent with the results of previous studies. However, Lange and associates (see Mandal *et al.*, 1977*b*) found deposits of immunoglobulins and electron-dense material in the glomeruli by immunofluorescence microscopy and electron microscopy, respectively. The glomerular finding suggesting an anti-GBM type of glomerulo-

Table 7-5
Results of Laboratory Studies in All Admissions in 1975

	6/5	7/12	7/25	9/11	9/21
Weight (g)		2860	2420		3260
Hematocrit	38.1	24.6			29.3
Serum Na^+ (mEq/liter)	136	128	137	126	139
Serum K^+ (mEq/liter)	4.5	4.4	3.9	4.3	4.4
Serum Cl^- (mEq/liter)		107	107	103	118
Serum CO_2 (mEq/liter)		16	20	22	10
BUN	13	13	10	8	25
Serum creatinine	0.3	0.4			
Serum albumin (g/100 ml)		1.0		0.9	1.0
Serum total protein (g/100 ml)	2.8	2.6		5.3	3.6
Cholesterol	268			230	
Urinary protein	++++	+++	+++	++	++++
Urinary sediment	4–50 RBC/HPF				

Source: From Mandal *et al.* (1977*b*), reproduced through the kind permission of the editor of *Human Pathology*.

nephritis may be supported by the observation of excessive amounts of basement membrane material (antigen) in the urine of eight patients with the Finnish type of congenital nephrotic syndrome. The latter observation suggests an abnormal reaction of glomerular basement membrane in congenital nephrotic syndrome. The findings of thrombi or fibrin fibers in the peritubular capillaries, veins, and

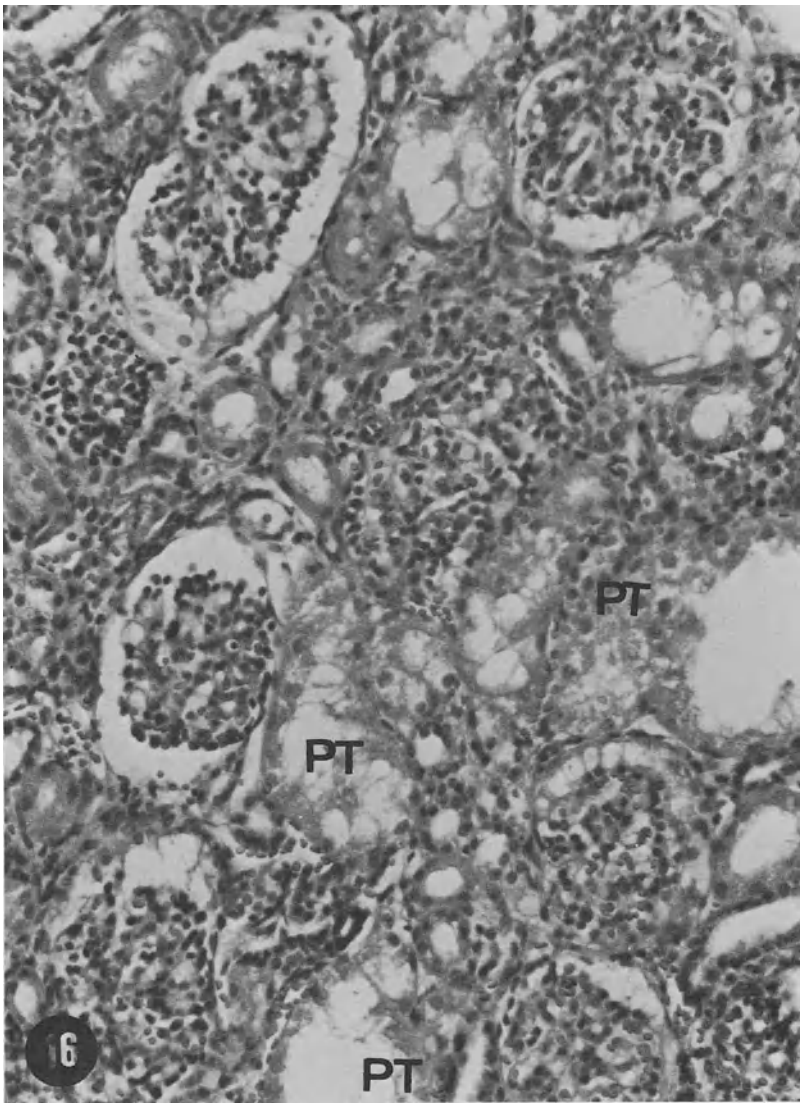


Fig. 7-16. Glomeruli show clusters of nuclei on the outer aspect of the tufts. These nuclei are the nuclei of the epithelial cells. The capillary loops are scarcely discernible. The large Bowman's spaces are apparently due to the small size of the glomerular tufts. Proximal tubules (PT) reveal vacuolated (cystic) change (H & E, ×100).

interstitium in our own study support this view. Some authors, in an attempt to explain the autoimmune type of glomerulonephritis in neonatal nephrosis, have suggested some type of immunological problem in the mother. Thus, an immune reaction may occur in the mother and her antibody by transplacental passage may be directed against GBM of the fetus kidney *in utero*.

The arteriolar abnormalities found in our study may be explained by the same mechanisms which account for changes in the glomeruli or tubules. These arteriolar changes are most likely to develop *in utero,* since the occurrence of such massive changes within 1 month after birth is inconceivable. It remains to

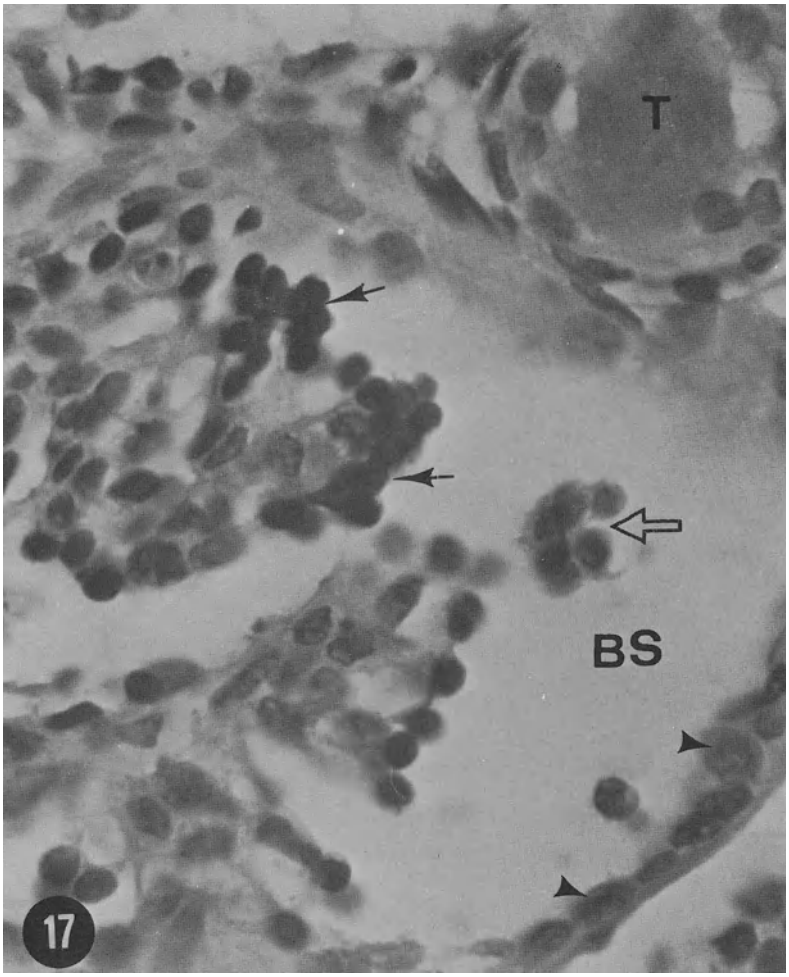


Fig. 7-17. Magnified view of a glomerulus reveals large epithelial nuclei (dark arrows), thin glomerular basement membranes, large Bowman's space (BS), and Bowman's epithelial nuclei (arrowheads). A detached fragment of a glomerulus (open arrow) and thrombus (T) in a periglomerular arteriole are shown (H & E, ×400).

be determined whether or not the nephrotic syndrome is produced by glomerular lesions alone or combined lesions in the glomeruli, tubules, and arterioles.

Pathogenesis of Congenital Nephrotic Syndrome

The pathogenesis of congenital nephrotic syndrome is still unclear. Immunofluorescence microscopy study has failed to demonstrate an immune mechanism in most cases. In rare cases, deposits of immunoglobulins were found. The available data suggest that an immunological mechanism may be implicated to explain some of the glomerular abnormalities.

RENAL AMYLOIDOSIS AND NEPHROTIC SYNDROME

Renal amyloidosis is rare among renal diseases encountered in the current practice of nephrology; the decreased incidence parallels the effective treatment

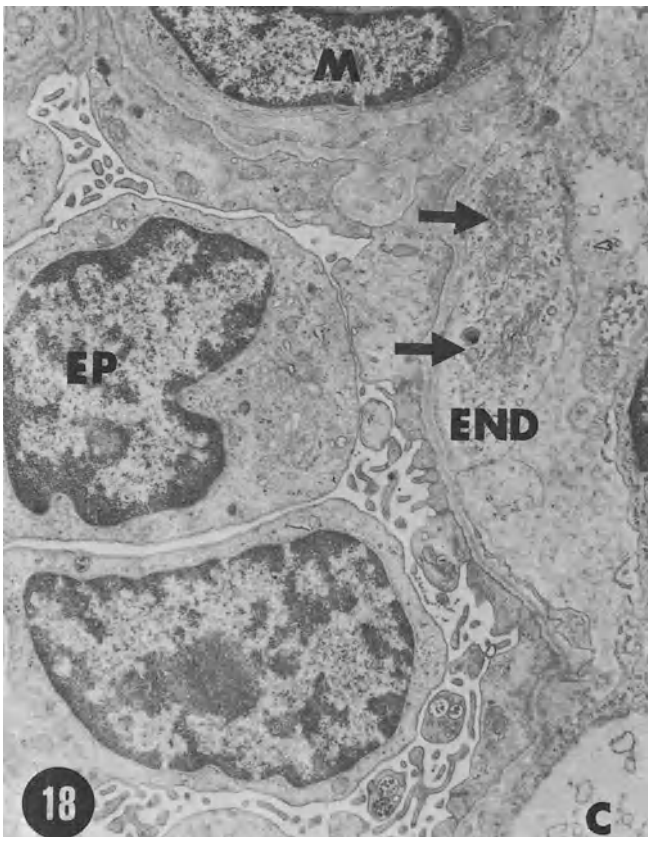


Fig. 7-18. Electron microscopy confirms thin glomerular basement membrane and large nuclei of the epithelial cells (EP). Endothelial cells (END) reveal prominent Golgi complexes (arrows) and cystic change (C). Mesangial cell (M) is shown (UA + LC, ×6000).

of tuberculosis and chronic pyogenic infections. The incidence of renal amyloidosis as a cause of nephrotic syndrome varies in different series; however, in general, it should be no higher than 1 to 2%. In our own series of EM studies of renal biopsies from 100 adults with asymptomatic heavy proteinuria and nephrotic syndrome during the last 5 years, amyloidosis has been confirmed in two patients.

The causes of renal amyloidosis are listed in Table 7-6. When amyloid is present in the kidney, it is most likely present in other organs, having also been observed, in decreasing order, in the spleen, liver, adrenal, and heart.

The diagnosis of amyloidosis is difficult to make, especially when light microscopy is used alone. The suspicion is most important; amyloidosis should be suspected when the material in the glomeruli and tubules is eosinophilic but PAS negative. The tissue must be studied by EM to confirm the diagnosis. Although LM study using various stains (e.g., methyl violet and congo red) and examination with ultraviolet light source has increased the positive diagnosis for amyloid significantly, it is not nearly as diagnostic as EM. When LM study reveals eosinophilic material distributed irregularly in the peripheral capillary loops of the glomeruli (Fig. 7-23a), it must be distinguished from diabetic glomerulosclerosis, focal glomerular sclerosis, and hepatic glomerulosclerosis. If the eosinophilic material is observed in the tubules and arterial vessels in addition to

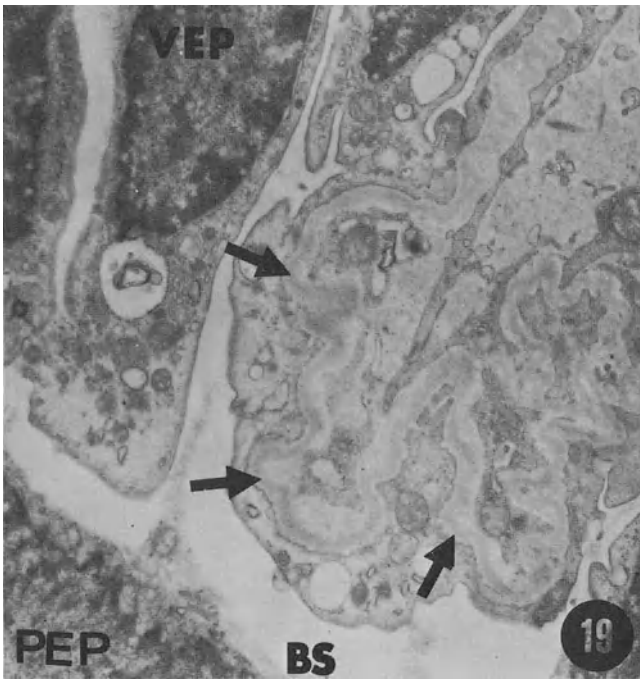


Fig. 7-19. Collapsed glomerular capillaries with electron-dense glomerular basement membranes (arrows). Visceral epithelial cells (VEP), Bowman's space (BS), and a parietal epithelial cell (PEP) are shown (UA + LC, ×8000).

the glomeruli, amyloidosis is the likely possibility. This eosinophilic material is PAS- and silver-negative. If an EM facility does not exist or EM study is pending, paraffin sections must be stained with congo red or methyl violet. When methyl violet- and congo red-stained sections are examined by polarizing microscopy, red and apple green birefringence appear, respectively, and in some cases, one or both stains may be negative. Thus, these special stains can in no way substitute for EM study.

There is a general agreement that EM study is the most important diagnostic aid in amyloidosis. However, unless there is a careful search, fine fibrils of amyloid can be missed under the electron microscope and a diagnosis of mem-

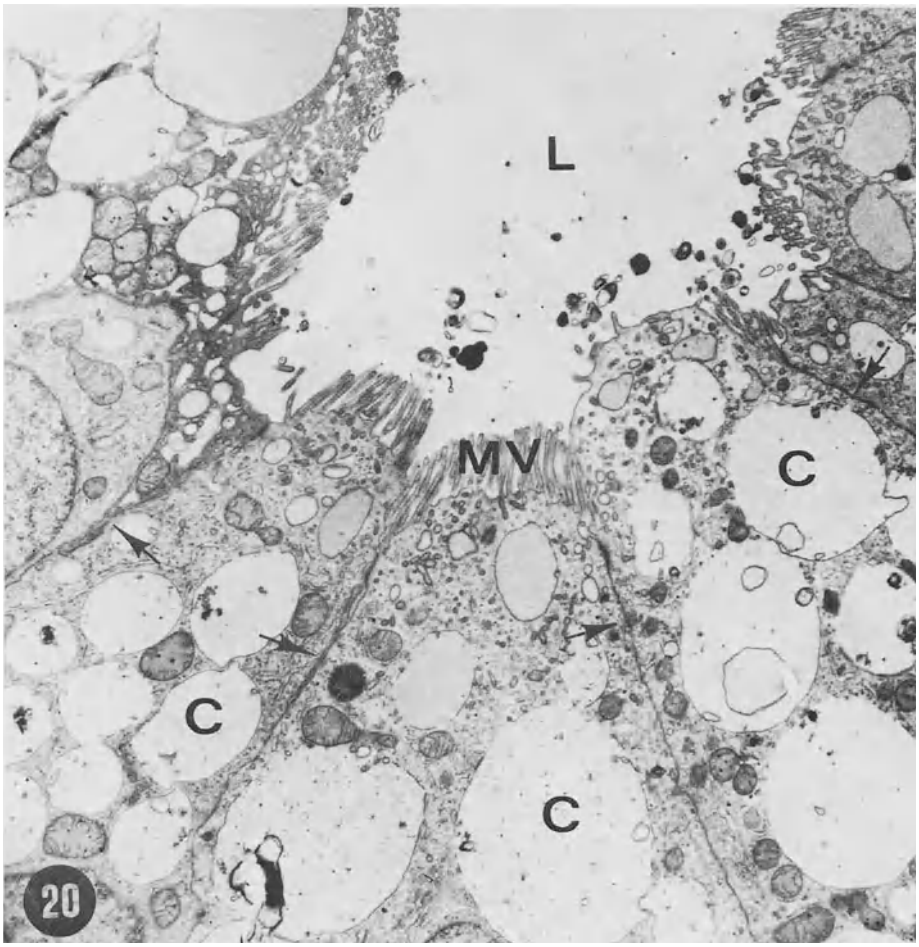


Fig. 7-20. Multiple cysts (C) within the cells of the proximal tubule. The cellular junctions (arrows) and the microvilli (MV) are generally intact. A few fragments of the cellular materials are seen within the lumen (L) of the tubule (UA + LC, ×10,000).

branous glomerulonephritis made in lieu of amyloidosis. The author studied two separate renal biopsies in great depth 3 years apart by LM and EM from a patient with IgA myeloma and amyloidosis. These studies are discussed in detail in Chapter 12. However, it should be mentioned that LM studies in both biopsies were unconvincing, even using congo red stain. Amyloidosis was suspected in the EM study of the first biopsy without prior knowledge of the clinical materials (Figs. 12-10 and 12-11). Amyloid fibrils were clearly discernible in the EM study of the second biopsy (see Figs. 12-12 and 12-13), for it is not at all difficult to diagnose amyloid in the advanced stage by EM study (Figs. 7-23b, 7-23c, and 7-24). In subsequent renal biopsy study in the same patient, congo red stain unequivocally demonstrated amyloid.

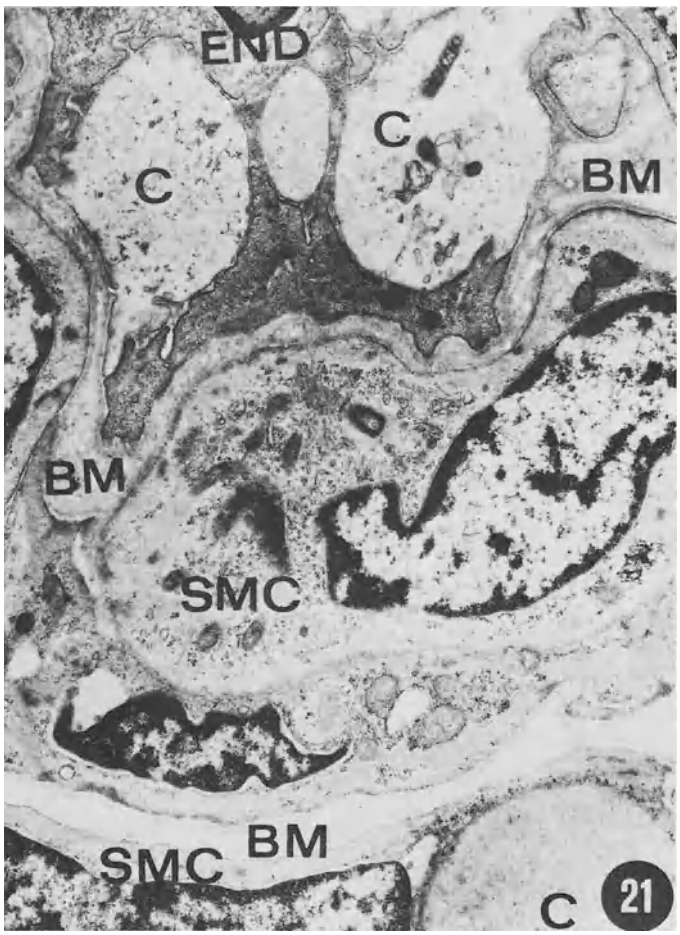


Fig. 7-21. Electron micrograph of a large arteriole reveals cysts (C) within the endothelial cells (END) and smooth muscle cells (SMC). Basement membrane (BM) is seen between END and SMC and between individual SMC (UA + LC, ×18,000).

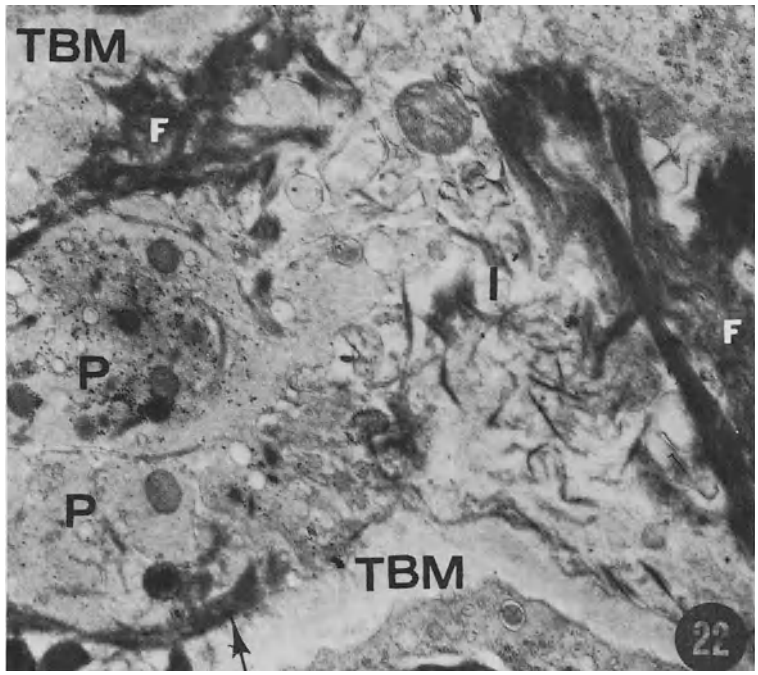


Fig. 7-22. Masses of fibrin (F) alone or surrounding platelet aggregates (P) (arrow) in the interstitium (I). Parts of tubular basement membranes (TBM) are seen (UA + LC, ×20,000).

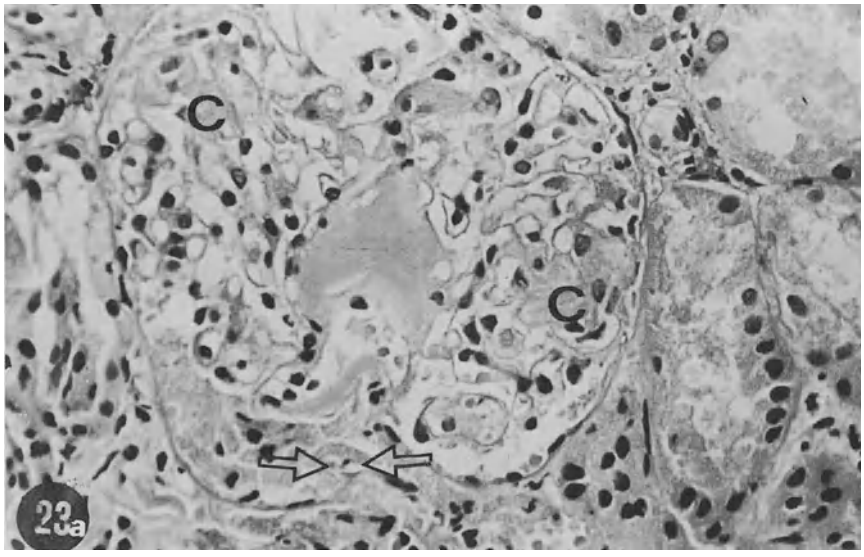
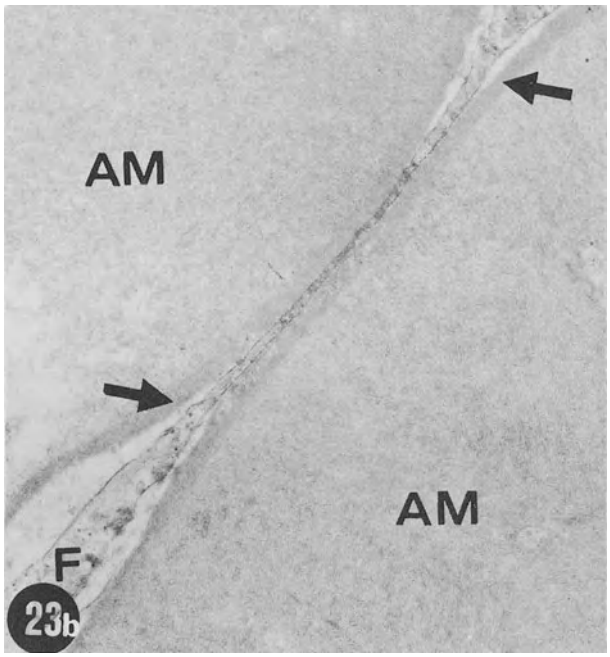


Fig. 7-23. (a) In this glomerulus, note the homogeneous eosinophilic material in the central part and vascular pole, and irregularly distributed in the peripheral capillary loops (C). Glomerulotubular communication (opposing arrows) can be seen (H & E, ×200). (b) The contents of the two adjacent tubules are completely replaced by amyloid material (AM). The basement membranes of the tubules (arrows) are necrotic. Fibroblasts (F) can be seen between the two tubules (UA + LC, ×12,000). (c) Magnified view of one of the two tubules in b clearly shows the amyloid fibers (UA + LC, ×36,000).



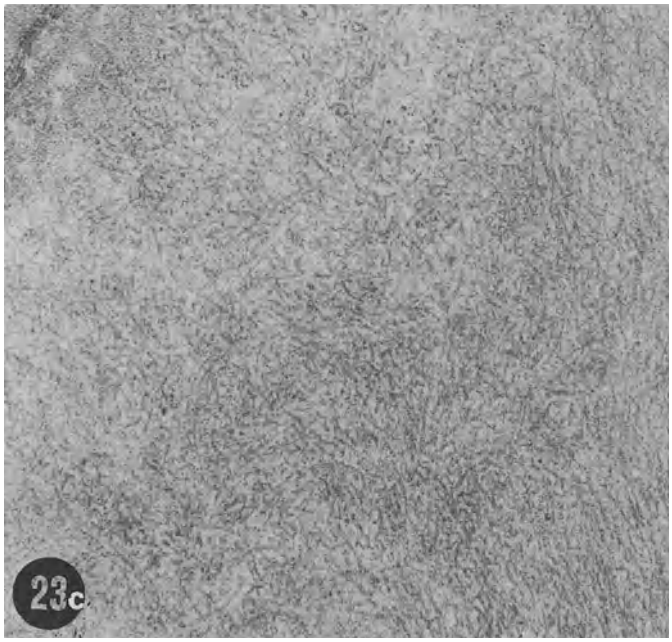


Fig. 7-23. *(Continued)*

Table 7-6
Etiology of Renal Amyloidosis

Type	Total number of cases
Primary amyloidosis	10
Familial Mediterranean fever	6
Myeloma	1
Secondary amyloidosis	
Tuberculosis	10
Osteomyelitis	6
Bronchiectasis	4
Rheumatoid arthritis	4
Crohn's disease	2
Chronic renal infection	1
Others	4

Source: From Triger and Joeker (1973), by the kind permission of D. R. Triger, M.D., The Royal Hospital, Sheffield, England, and the editor of the *Quarterly Journal of Medicine*.

From the clinical standpoint, it is important to suspect and establish the diagnosis of amyloidosis for the following reasons:

1. Since LM study may be unremarkable, especially in the early stage, a diagnosis of minimal lesion disease or lipoid nephrosis may be made erroneously.
2. If the foregoing diagnosis is made, treatment may be instituted with corticosteroids, and there is some evidence to suggest that corticosteroids may be harmful in amyloidosis.
3. Amyloidosis is an irreversible and slowly progressive disease.
4. Improvement of renal function after treatment with melphalan, especially when amyloidosis follows myeloma, has been reported.
5. Detection of renal amyloidosis may warrant a search for occult infection, e.g., tuberculosis, tumor, or myelomatois.

RENAL INVOLVEMENT IN DIABETES MELLITUS

SYNONYMS. Diabetic renal disease, diabetic nephropathy, diabetic glomerulosclerosis, Kimmelstiel–Wilson lesion.

There are three separate mechanisms by which the kidneys may become involved in diabetes mellitus: (1) diabetic glomerulosclerosis *per se*, (2) acute and chronic pyelonephritis, and (3) arteriolosclerosis and arteriosclerosis. The term diabetic nephropathy is applied loosely to denote diabetic glomerulosclerosis, although the ideal terminology for renal lesions in diabetes mellitus would be diabetic nephrosclerosis rather than diabetic glomerulosclerosis. Except in the

early stage of diabetes mellitus, the preceding triad is observed most frequently in biopsy or autopsy studies of the kidney from long-standing diabetes mellitus.

Diabetic nephropathy was eloquently described by the late Dr. Paul Kimmelstiel in 1966, and more recently highlighted by Churg and Dachs (1975). Therefore, our intention is not to burden the readers with another repetitive version of diabetic nephropathy. In this section, we shall attempt to clarify some obscure aspects of diabetic renal disease, including (1) a brief summary of the pathology of diabetic glomerulosclerosis, (2) the relationship of diffuse diabetic glomerulosclerosis to nodular diabetic glomerulosclerosis, (3) the value of renal biopsy in the prediabetic state, (4) the clinical significance of arteriolar lesions or arteriololslcerosis, and (5) a clinician's view of renal tissue diagnosis in diabetes mellitus.

The Pathology of Diabetic Glomerulosclerosis

There are two basic changes observed in the glomerulus: sclerosis and hyaline deposits. The sclerosis is defined as an increase of basement membrane-like material in the mesangium with secondary encroachment upon peripheral

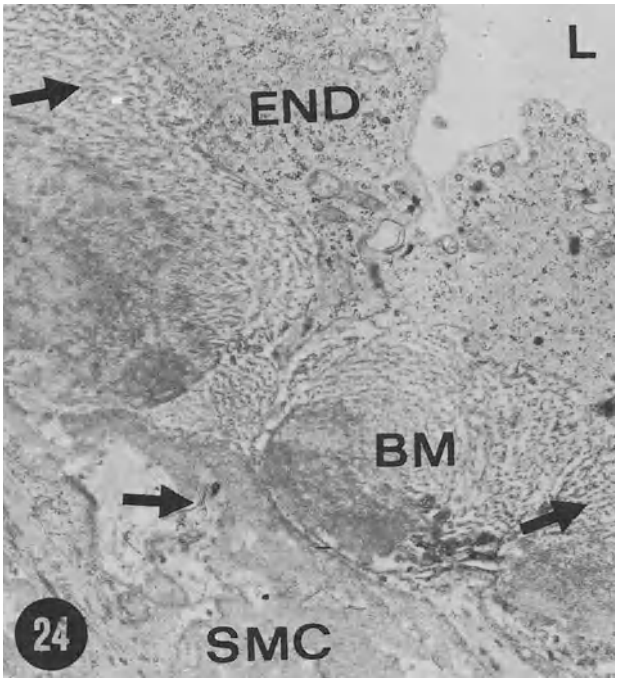


Fig. 7-24. In this large arteriole, basement membrane (BM) and smooth muscle cells (SMC) are largely replaced by amyloid deposits. The amyloid fibers are shown (arrows). Endothelial cells (END) are atrophic. Lumen (L) of the arteriole can be seen (UA + LC, ×8200).

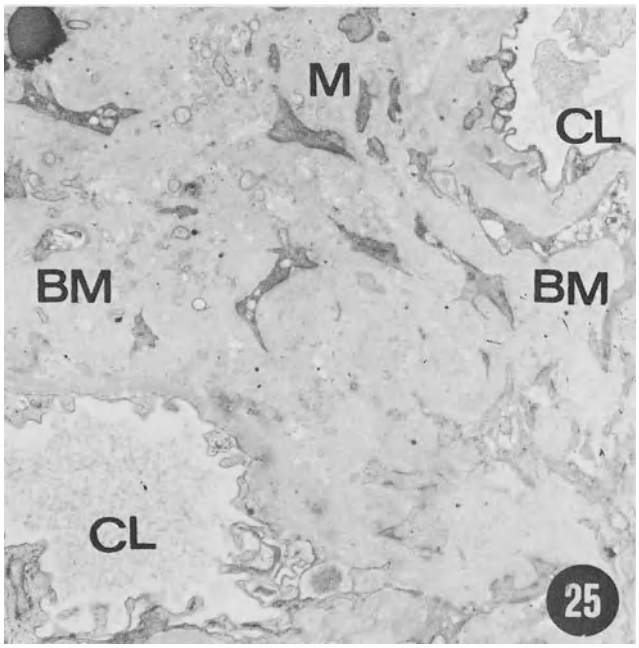


Fig. 7-25. Masses of basement membrane-like materials (BM) in the mesangium (M) with encompassment of the capillary loops (CL) (UA + LC, ×4000). From the renal biopsy of a 46-year-old white female who presented with heavy proteinuria and had a 15-year history of diabetes mellitus.

capillaries and thickening of peripheral capillary basement membrane (Fig. 7-25).* The process is discrete and conspicuous in nodular type (Figs. 7-26 and 7-27), and less definitive in diffuse type (Figs. 7-28 and 7-29). In the latter, thickness of the peripheral capillary basement membrane varies from 2500 (normal) to 25,000 Å or more (marked thickening). The lumen is usually decreased in diameter owing to thickened capillary walls and excessive mesangial matrix. The frequency of glomerulosclerosis in diabetes mellitus varies from series to series. It has been reported to be as low as 7.6% and as high as 53%. In our opinion, the variation can be attributed to three important factors: (1) the technique used to study renal tissue, i.e., only LM or both LM and EM; (2) the duration of the disease at the time of study of the kidney; and (3) a difference in the interpretation of findings. The diffuse diabetic glomerulosclerosis characterized by mild thickening of peripheral capillary loops may be misinterpreted as minimal lesion disease or early membranous glomerulonephritis by LM study. EM study is therefore recommended.

The second process, hyaline deposition, is superimposed upon sclerosis and consists of homogeneous acidophilic, proteinaceous, often lipid-rich material (Fig. 7-27).

* Figures 7-25 through 7-36 demonstrate a variety of renal changes in diabetes mellitus.

The nodular diabetic glomerulosclerosis shown in Fig. 7-27 resembles focal glomerular sclerosis, particularly because of the intracapillary eosinophilic material (hyaline material) and foam cells observed. The mere history of diabetes mellitus is not a decisive factor since diabetic kidney may be associated with another glomerular disease. Using LM study alone, diabetic glomerulosclerosis can be distinguished from focal glomerular sclerosis by (a) involvement of almost all glomeruli in diabetes mellitus in contrast to many normal glomeruli in focal glomerular sclerosis; (b) arteriosclerosis, especially of the arterioles at the vascular pole of the glomerulus in diabetes mellitus (Fig. 7-27); and (c) thickening of tubular and capillary basement membranes in diabetes mellitus but less so in focal glomerular sclerosis.

EM study may fail to distinguish between the two conditions because both may exhibit electron-dense deposits and excessive mesangial matrix. Protein deposit on the endothelial aspect of GBM in diabetes mellitus has been described by Churg and Grishman (1975). We have found electron-dense deposits in the subendothelial locations of many glomerular capillaries in one of our patients with a 10-year history of diabetes mellitus (Figs. 7-30 and 7-31). The distinguishing ultrastructural features of the two conditions consist of a pronounced thickening

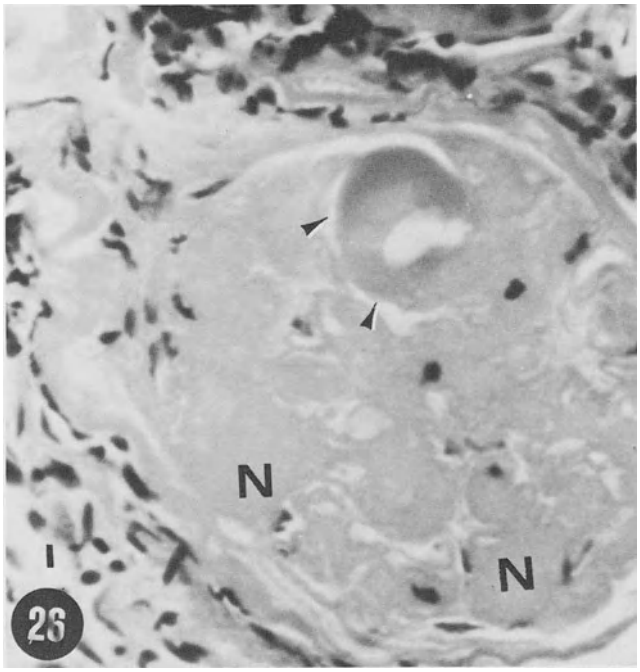


Fig. 7-26. The glomerular tufts have been transformed into several nodules (N). An isolated markedly thickened capillary loop has assumed an appearance sometimes described as "fibrin cap" (arrowheads) (PAS, ×400). From the same patient as in Fig. 7-25.

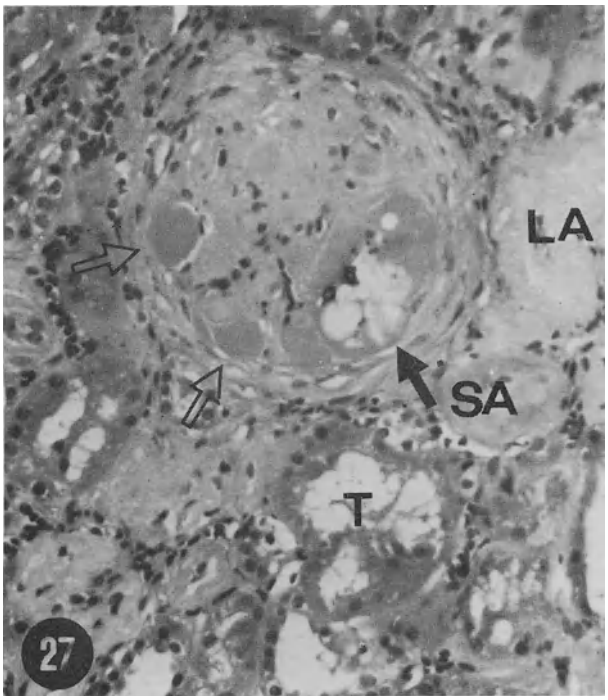


Fig. 7-27. The glomerulus reveals several nodules as well as collections of homogeneous material (open arrows) which have been described as capsular drop, fibrin cap, and so on. The foamy appearance (dark arrow) is due to accumulation of lipid droplets within endothelial cells (forming so-called endocapillary foam cells which are a rare finding in diabetic nephropathy but are commonly observed in focal glomerular sclerosis). The foamy appearance in the tubules (T) also is due to accumulation of lipid droplets. Thickening of a periglomerular large arteriole (LA) and a small arteriole (SA) is shown (H & E, ×100).

of tubular basement membrane and replacement of parts of the tubules by basement membrane-like materials or deposits in diabetes mellitus, but not in focal glomerular sclerosis (Fig. 7-32).*

Relationship of Diffuse to Nodular Diabetic Glomerulosclerosis and Their Specificity

The interrelationship of the two types of diabetic glomerulosclerosis is still largely unclear. It is completely unknown why in some instances only nodules are found (Fig. 7-26), whereas in others only diffuse glomerulosclerosis is found, while in most cases the mixed form is observed (Fig. 7-28).

Opinion is divided concerning the sequential development of one form from the other. Some believe that the thickening of peripheral capillary basement membranes, i.e., diffuse glomerulosclerosis, precedes laying of basement membrane-like material in the mesangium, i.e., nodular glomerulosclerosis. Others hold that the opposite is true. In support of the former hypothesis, it may be argued that glomerular capillaries undergo a change similar to that in capillaries

* Figures 7-32, 7-34, and 7-36 are from the renal biopsy of a 65-year-old white male who presented with mild proteinuria. Diabetic glomerulosclerosis was suspected from EM study of the renal biopsy. This morphological diagnosis was supported by diabetic type results of a glucose tolerance test 18 months later.

elsewhere in the body. The peripheral capillary lesions are considered specific for diabetes mellitus. Therefore, diffuse thickening of basement membranes in glomeruli, akin to thickening of basement membranes in the peripheral capillaries elsewhere, must be regarded as the basic pathological lesion of diabetes mellitus. In this context, the formation of nodules is the sequela of the advanced stage of diffuse glomerulosclerosis, or diffuse thickening of basement membranes of other capillaries. Churg and Dachs (1975) believe that initial changes occur in the

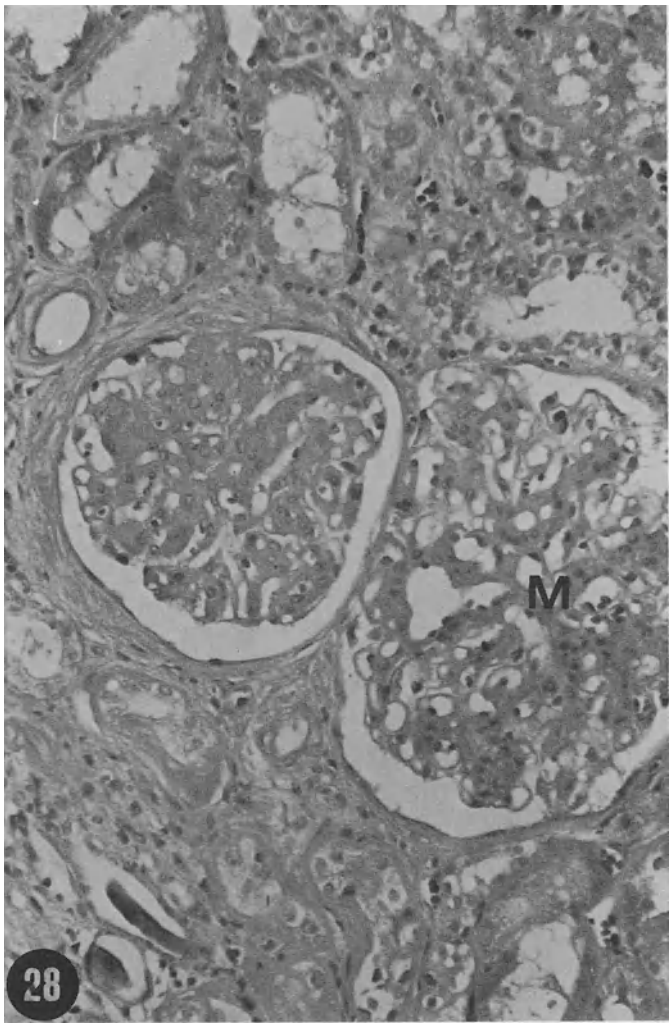


Fig. 7-28. Two adjacent glomeruli reveal disparate changes. Left glomerulus: nodular sclerosis; right glomerulus: diffuse thickening of the peripheral capillary loops with tendency toward nodularity. The mesangium (M) is prominent. Some tubules show foamy appearance (upper central), whereas others reveal casts within the lumen (lower left) (H & E, ×120). From a 56-year-old white male with a 16-year history of diabetes mellitus. His 24-hr proteinuria was 0.6 to 1.2 g and 24-hr creatinine clearance was approximately 40 ml/min.

mesangium and in the capillary basement membrane. Basement membrane-like materials and matrix keep accumulating in the mesangium. Eventually, accumulation of the materials reaches the point at which true nodules are formed in the lobular centers (Fig. 7-33).

There is a significant functional difference between the two types of diabetic glomerulosclerosis, inasmuch as the nephrotic syndrome is closely related to the diffuse type and and not to the nodular lesion.

Kimmelstiel (1966) believed that the nodular type is virtually the pathognomonic diabetic renal lesion and that the diffuse type is nonspecific. Churg and Dachs (1975) concur with Kimmelstiel's notion. In the absence of EM, based on the reasons stated earlier, it would be a mistake to rely on the LM observation of diffuse glomerulosclerosis as a specific diabetic renal lesion.

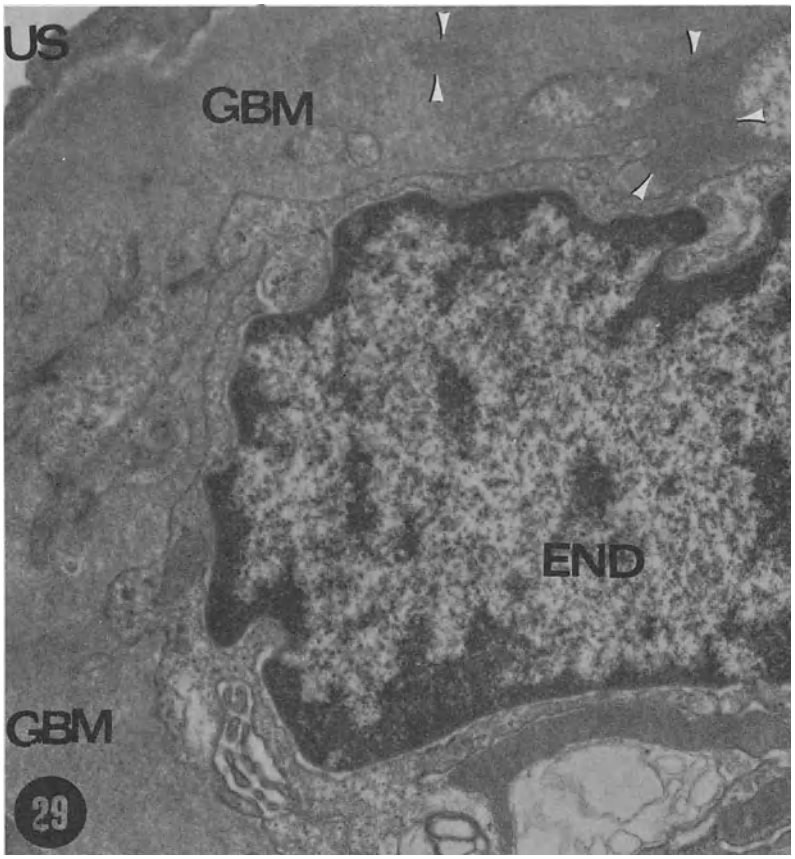


Fig. 7-29. Diffuse diabetic glomerulosclerosis: diffuse uniform thickening of the glomerular basement membrane (GBM). This is differentiated from membranous glomerulonephritis by the absence of spikes and extramembranous deposits. Small discrete electron-dense deposits (arrowheads) are shown within the GBM. Endothelial cells (END) and urinary space (US) are shown (UA + LC, ×22,000).

Although the glomerular lesions are more common and more severe in frank diabetes mellitus, they also appear in people with latent diabetes mellitus and even in those with prediabetes. The glomerular lesions in diabetes mellitus may be focal or generalized and segmental or diffuse. Even so, ultrastructurally, the thickening of glomerular basement membrane may be segmental or diffuse and vary from one glomerulus to another. Segmental thickening of glomerular basement membrane has been reported in the prediabetic state. EM study of renal biopsy is very useful in recognizing and establishing the clinical prediabetic state. EM has helped to diagnose the prediabetic condition by the findings of segmental

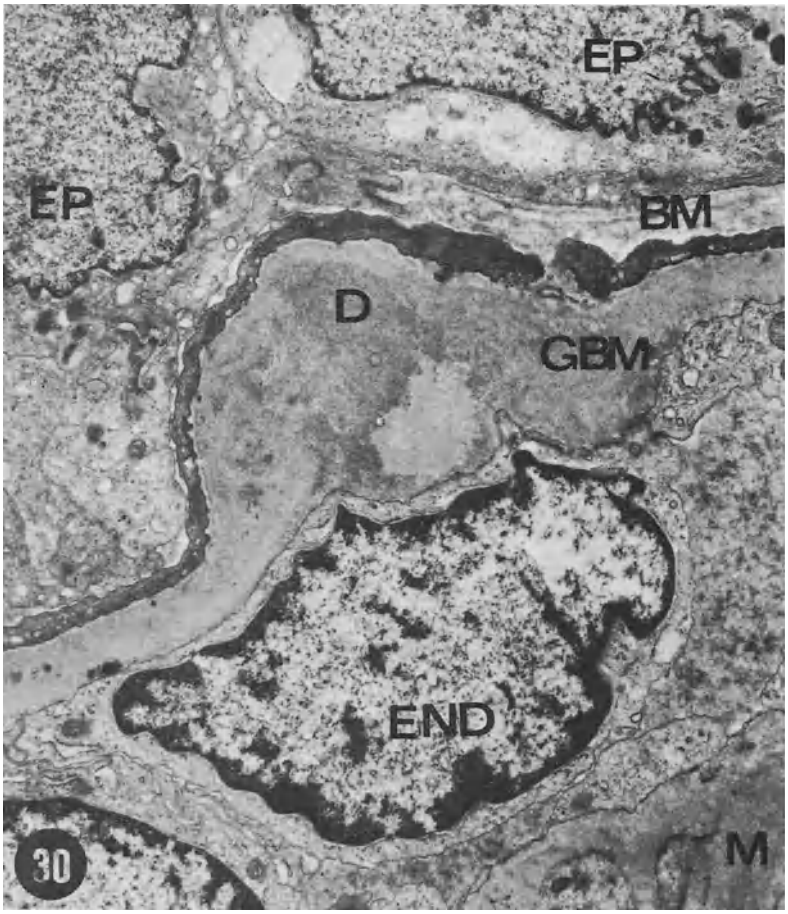


Fig. 7-30. Mild thickening of the glomerular basement membrane (GBM) and electron-dense deposit (D) in the subendothelial location. Hyperplasia of epithelial cell (EP), excessive basement membrane-like materials (BM), endothelial cells (END), and increase in mesangial matrix (M) are shown (UA + LC, ×20,000).

thickening of glomerular basement membrane (Fig. 7-34) and thickening of peritubular capillary basement membrane (Fig. 7-32) as the cause of unexplained proteinuria in four patients in whom clinical or laboratory evidence of diabetes mellitus did not develop until several months later. At the time of the renal biopsy, results of the glucose tolerance test were normal in three of our patients; the fourth patient had a 4-hr hypoglycemic glucose value of 52 mg/100 ml. Eighteen months later, two patients had glucose tolerance tests compatible with those for diabetes mellitus; the other two patients had diabetic glucose tolerance curves and glycosuria (for details, see Muehrcke *et al.*, 1969).

Clinical Importance of Arteriolar Lesions or Arteriolosclerosis

In the kidney, small arteries, large or small arterioles, and peritubular capillaries become severely involved in diabetes mellitus (Figs. 7-32 and 7-35). Marked thickening of the basement membrane between endothelial cell and smooth muscle cell in the small renal arteriole (Fig. 7-36) and granular deposit surrounding the pericyte in the peritubular capillary (Figs. 7-32 and 7-36) have

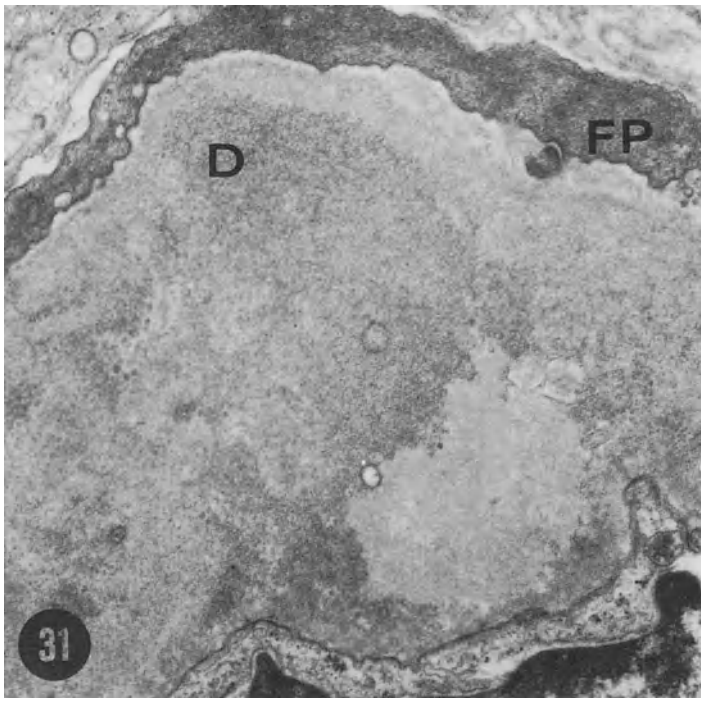


Fig. 7-31. Magnified view in which subendothelial deposit (D) is clearly observed. Note fusion of foot processes (FP) (UA + LC, ×40,000). From the renal biopsy of a 63-year-old white male who is known to have had diabetes mellitus for 10 years. He presented with edema and was found to have 20 g protein/24-hr urine. He had a serum creatinine of 1.4 mg/100 ml.

been observed. These arteriolar and capillary changes have potential clinical significance, namely, a relationship to papillary necrosis; they may cause papillary ischemia and explain a high incidence of papillary necrosis in diabetes mellitus.

Clinician's View of Renal Tissue Diagnosis in Diabetes Mellitus

The one question that must be answered before planning renal biopsy in diabetes mellitus pertains to the rationale of renal biopsy study in this disease. Statistically, a patient with long-standing diabetes mellitus would demonstrate on renal biopsy the triad of nodular or diffuse diabetic glomerulosclerosis, acute and chronic pyelonephritis, and arteriolosclerosis. In addition, 5 to 20% of patients may exhibit another disease such as membranous glomerulonephritis, especially if the patient has heavy proteinuria despite declining renal function.

Of course, for us, these are interesting and valuable learning experiences, but what about the patient? After subjecting him/her to an invasive procedure associated with a higher incidence of postbiopsy complications and an expenditure of $400 to $500 to evaluate the renal biopsy, what are we going to offer the

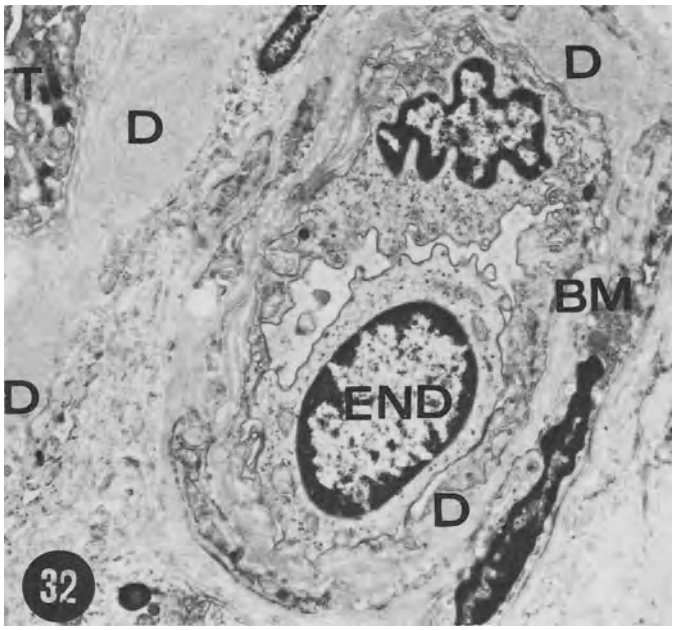


Fig. 7-32. Electron-dense deposits (D) are seen in the basement membrane of a tubule (T). A peritubular capillary demonstrates thickening of the basement membrane (BM) and segmental expansion of BM by electron-dense deposits. Endothelial cells (END) of the peritubular capillary are seen (UA + LC, ×6200).

patient? We know little about the correlation between the type of diabetic renal lesion and status of renal function; therefore, the course of the disease cannot be assessed accurately. Also, we do not know of any therapy to retard progression of diabetic renal disease. It is clear then that the practical gain from renal tissue diagnosis is not sufficient to warrant routine renal biopsy study in diabetes mellitus.

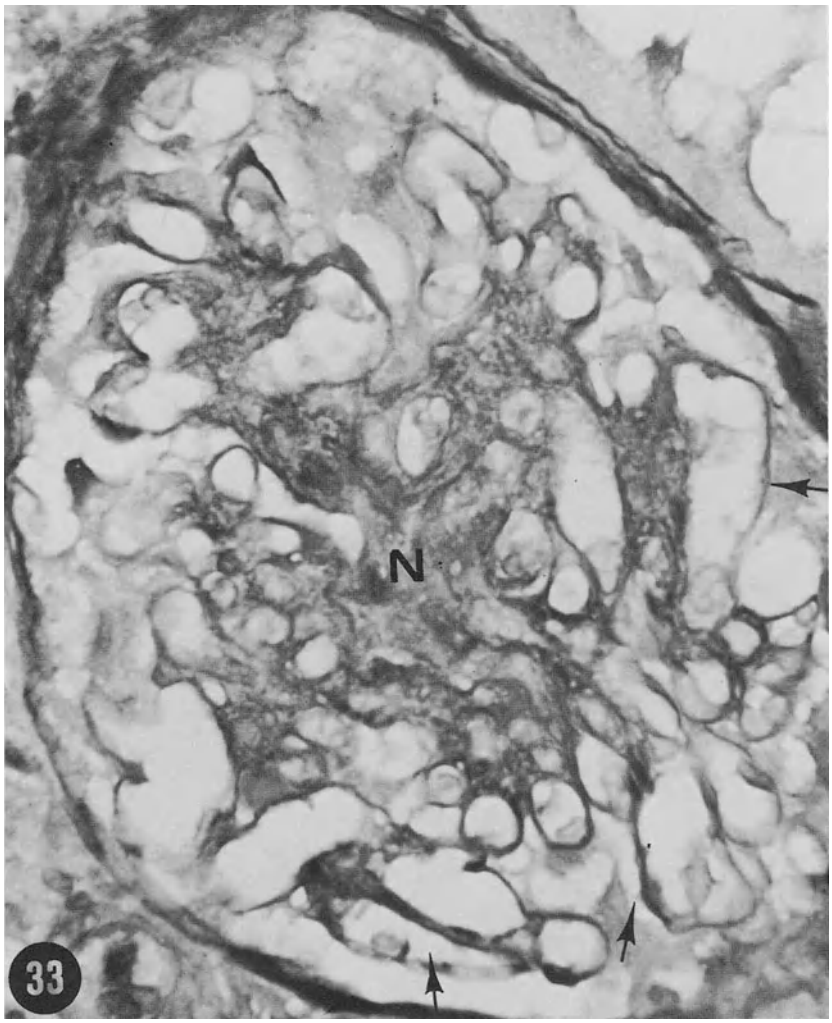


Fig. 7-33. This glomerulus demonstrates nodular sclerosis (N) in the central (or mesangial) portions of the glomerular tufts and thickening of the peripheral capillary loops (arrows). These findings support the hypothesis of Churg and Dachs that sclerotic change starts in the mesangium and the sclerotic materials keep accumulating and thereby form nodules (PAS, ×400).

Nephrotic Syndrome in Chronic Interstitial Nephritis (Pyelonephritis)

Chronic interstitial nephritis (pyelonephritis) is a common morphological diagnosis on postmortem examination; only rarely has it been reported to produce heavy proteinuria or nephrotic syndrome. Chronic pyelonephritis as a cause of nephrotic syndrome seems to be more common in the Eastern Hemisphere than the Western world; for example, chronic pyelonephritis was found by biopsy and/or autopsy studies in 15% of a total of 174 cases of nephrotic syndrome in a series studied by Vaishnava and colleagues (1968) in India. However, in no studies has an etiology of nephrotic syndrome been determined. We have found, by electron microscopy study, glomerular changes consistent with foot process fusion disease (lipoid nephrosis) in three patients with chronic pyelonephritis and nephrotic syndrome. This ultrastructural glomerular abnormality might explain nephrotic syndrome observed in chronic interstitial nephritis (chronic pyelone-

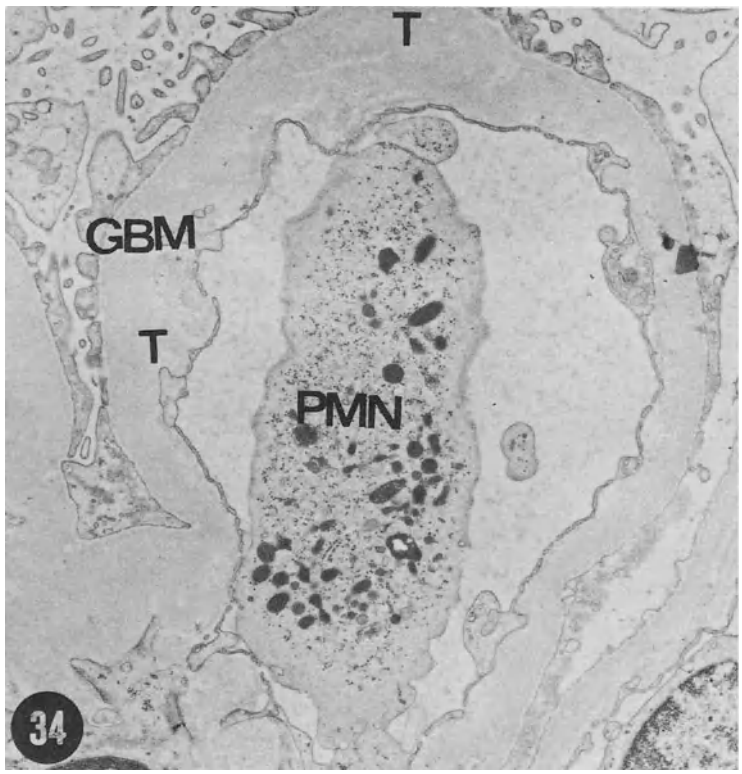


Fig. 7-34. Irregular and segmental thickening (T) of the basement membrane (GBM) of a glomerular capillary. A neutrophil leukocyte (PMN) is seen within the lumen of the capillary (UA + LC, ×8400).

phritis). The details of one of the three patients studied are included here [from Mandal *et al.* (1977*a*), by the kind permission of the editor and publisher of the *American Journal of the Medical Sciences*]:

Patient L.B., a 53-year-old white male, was admitted to Oklahoma City Veterans Administration Hospital in January 1974 with a 5-day history of dyspnea and pedal edema. He showed signs of congestive cardiac failure and had normal blood pressures. Laboratory data in this patient (#1) and two other patients are shown in Table 7-7. A chest x-ray showed congestive changes bilaterally; an ECG demonstrated an old anteroseptal myocardial infarction; a selective left renal arteriogram revealed cortical atrophy and caliceal clubbing; and bilateral renal venography exhibited patency of both renal veins. Bilateral retrograde pyelography revealed clubbing of the lower pole calyx and a "ring shadow" (a characteristic radiological sign of papillary necrosis) in the upper pole calyx of the left kidney, faintly visualized calyces in the right kidney, asymmetry in size of the kidneys, with the left smaller than the right by more than 1 cm, and a stone in the left ureter. Symptoms and chest x-ray findings cleared with digitalization and diuretic therapy. A percutaneous right renal biopsy was performed, and renal tissue was studied by LM, EM, and IFM. Of 20 glomeruli, LM study showed 18 to be normal and two hyalinized. The consistent changes were periglomerular fibrosis, a scarcity of tubules, some atrophic and dilated tubules, and focal interstitial infiltrates of inflammatory cells. Some dilated tubules contained necrotic material impregnated with polymorphonuclear leukocytes and round cells. Small arterioles were observed and appeared normal. EM of two glomeruli showed generalized fusion of epithelial foot processes in most of the capillaries (Fig. 7-37). The glomerular basement membranes in some capillaries were slightly irregular and tortuous;

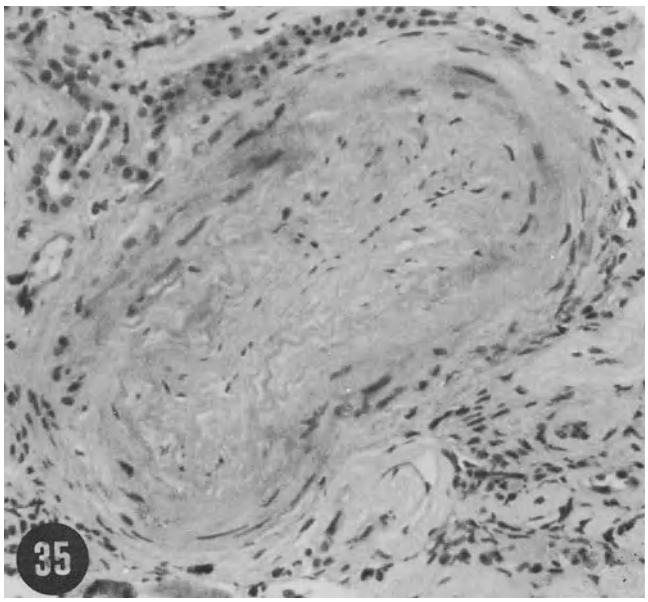


Fig. 7-35. Marked thickening of the small artery due to excessive basement membrane-like matrix. Marked atrophy of tubules, interstitial fibrosis, and pleomorphic cellular infiltrates are seen (H & E, ×320). From the renal biopsy of the same patient as in Fig. 7-27 and 7-28.

endothelial cells in some capillaries were slightly enlarged and contained vacuoles. There was remarkable absence of hypercellularity and electron-dense deposits. The interstitium revealed severe infiltration by lymphocytes, plasma cells, and fibroblasts. IFM of renal tissue stained with fluorescein-conjugated antisera against human IgG, IgA, IgM, IgE, C3, and fibrinogen was negative. Because of the EM diagnosis of lipoid nephrosis, negative staining for all immunoglobulins, and persistent massive proteinuria (1 month after discharge, 24-hr proteinuria was 17.9 g), the patient was placed on oral prednisone 80 mg daily. After 2 weeks, his 24-hr proteinuria fell to 2.4 g; after 4 weeks of treatment, his laboratory studies revealed proteinuria 1.1 g/24 hr, serum urea nitrogen 54 mg/100 ml, and serum albumin 4.2 g/100 ml. He expired suddenly before the next visit and no autopsy was performed (see Chapter 10 for details on this patient's renal biopsy).

Another patient, a 14-year-old black female (patient #3 in Table 7-7), showed partial improvement after corticosteroid therapy. She developed renal failure, underwent bilateral nephrectomy, and was subjected to chronic hemodialysis. Nephrectomy specimen studied by LM showed normal glomeruli and severe

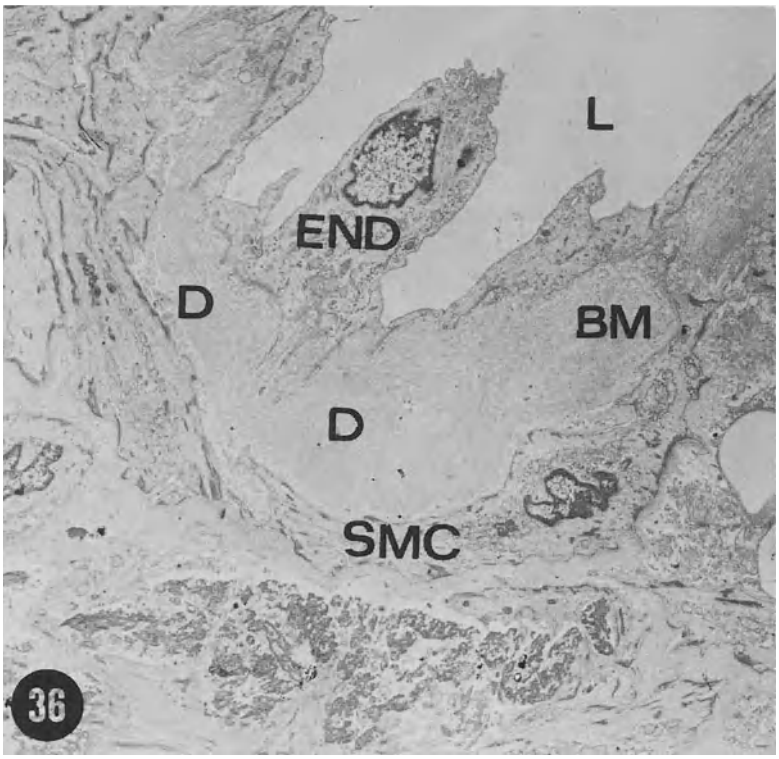


Fig. 7-36. Light electron-dense deposits (D) within the basement membrane (BM) of the arteriole between endothelial cell (END) and smooth muscle cell (SMC). The lumen of the arteriole (L) is shown (UA + LC, ×3400). This type of electron-dense deposit in the arteriole has also been observed in focal glomerular sclerosis and membranous glomerulonephritis (for more details see Chapter 12).

Table 7-7
Laboratory Information in Three Patients with Chronic Pyelonephritis and Nephrotic Syndrome

	Patient #1	Patient #2	Patient #3
Urinary studies			
Protein excretion (g/24 hr)	8.2–21.7	6.1–8.2	23.7
Sediment			
WBC/HPF	Numerous	4–5	10–20
RBC/HPF	0–3	Numerous	0–3
Cultures	*Proteus mirabilis*	Negative	Negative
Serum studies			
Total protein (g/100 ml)	4.1	6.2	4.8
Albumin (g/100 ml)	0.7	3.0	1.1
Cholesterol (mg/100 ml)	247	245	500
Urea nitrogen (mg/100 ml)	63	64	24
Creatinine (mg/100 ml)	5.2	5.6	1.4
Antinuclear antibody	Negative	Negative	Negative
ASO titer	Normal	Normal	Normal

Source: From Mandal *et al.* (1977*a*), by the kind permission of the editor and publisher of the *American Journal of the Medical Sciences*.

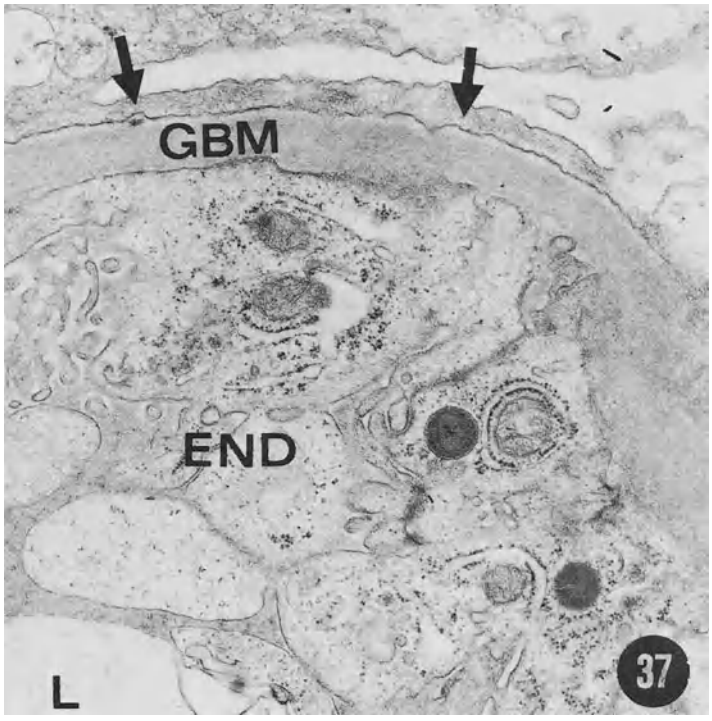


Fig. 7-37. This glomerular capillary shows generalized fusion of foot processes (arrows) and normal basement membrane (GBM). Endothelial cell (END) has prominent rough-surfaced endoplasmic reticulum. Lumen (L) of the capillary is shown (UA + LC, ×15,000).

progression of the tubulointerstitial changes. Two glomeruli from nephrectomy specimens studied by EM revealed diffuse fusion of foot processes in some capillaries but normal foot processes in others (for more information, see Chapter 10).

Although it is an established fact that heavy proteinuria (nephrotic syndrome) occurs in association with chronic pyelonephritis, the mechanism of this severe proteinuria remains unclear. Vaishnava *et al.* (1968) found good correlation between the severity of histopathological changes (histological grading) and the degree of proteinuria (shown in Table 7-8).

The possibility of coexistent minimal or nil lesion disease has been suggested in all earlier studies, but this has been neither confirmed nor ruled out by EM and IFM. Our own study was the first to show that foot process fusion disease or lipoid nephrosis may be the cause of nephrotic syndrome in chronic pyelonephritis. In any case, it is still undetermined whether the chronic interstitial nephritis (pyelonephritis) has produced lipoid nephrosis through an unidentified mechanism, whether the lipoid nephrosis has predisposed the patients to chronic interstitial nephritis, or whether the two disparate entities have coexisted.

Nephrotic Syndrome in Association with Hereditary Nephritis

Like chronic pyelonephritis, nephrotic syndrome occurs rarely in hereditary nephritis. Two separate series have described heavy proteinuria exceeding 3.5 g, indicating nephrotic syndrome in 5 of 15 patients with hereditary nephritis and deafness from six families and two of seven patients with hereditary nephritis from a single family. There is no specific histological change observed to account for the nephrotic syndrome in hereditary nephritis. In one series, renal tissues were studied by LM and EM. The LM findings were consistent with either end-stage renal disease or chronic pyelonephritis, whereas the EM study was nonspecific except in confirming the diagnosis of hereditary nephritis. Therefore, the cause of nephrotic syndrome in hereditary nephritis remains undetermined. Since most of the glomeruli were hyalinized and proteinuria did not remit after corti-

Table 7-8
Correlation between Histological Lesions and Proteinuria

Histological grading	Number of cases	24-hr proteinuria (g/24 hr)	
		Range	Mean
I	1	3.2	3.2
II	5	3.8–5.4	4.8
III	10	4.0–8.6	5.8
IV	12	5.8–9.4	7.2

Source: From Vaishnava *et al.* (1968), by the kind permission of the authors; Maulana Azad Medical College and Hospitals, New Delhi; and the editor of the *Journal of the Association of Physicians of India.*

costeroid therapy, the possibility of a coexistent renal pathology, e.g., lipoid nephrosis, which was observed in association with chronic pyelonephritis, is less likely or unlikely.

Nephrotic Syndrome during Pregnancy

The clinical picture of nephrotic syndrome during pregnancy may be variable and it must be distinguished from preeclampsia. The distinguishing features of nephrotic syndrome and preeclampsia are listed in Table 7-9.

Approach to the Patient with Nephrotic Syndrome during Pregnancy

Membranous GN is the most common pathologic lesion observed in nephrotic syndrome during pregnancy. The obstetrician and internist must be alerted to two important complications which concern nephrotic syndrome during pregnancy: (1) infection and (2) thromboembolic phenomena. The maternal and

Table 7-9
Differentiation of the Nephrotic Syndrome from Preeclampsia

	Nephrotic syndrome	Preeclampsia
Onset	Any trimester	After 20 weeks
Hypertension	Usually absent	Present
Proteinuria	Massive	Varying degrees
Hematuria	Microscopic hematuria may be present	Absent
Lipiduria	Frequent (oval fat bodies may be seen)	Absent
Edema	Usually marked	Variable
Serum lipids	Usually markedly elevated	Occasionally slightly elevated
Hypoproteinemia	Marked	Moderate
Glomerular filtration rate	Decreased, normal, or elevated	Decreased
Renal plasma flow	Decreased, normal, or elevated	Decreased
Serum protein electrophoresis	Decreased albumin, $alpha_1$ and gamma globulin, and antithrombin; increased $alpha_2$ and beta globulin; increased fibrinogen	Decreased albumin, beta and gamma globulin, increased $alpha_1$ and $alpha_2$ globulin; increased fibrinogen
Thyroid function	Decreased BMR,[a] decreased PBI,[b] decreased thyroxine binding globulin	BMR, PBI, and thyroxine binding usually increased (as in normal pregnancy)
Electron microscopy	Epithelial foot processes markedly distorted or lost, vacuolization of epithelial cytoplasm, thickening and spiking of basement membrane	Slight to moderate distortion of foot processes, endothelial swelling, vacuolization of endothelial and epithelial cytoplasm, subendothelial deposit, normal basement membrane

[a] Basal metabolic rate. [b] Protein-bound iodine.
Source: From Marcus (1963), reprinted through the kind permission of the editor of the *Obstetrical and Gynecological Survey*.

fetal prognoses are difficult to assess owing to the rarity of the syndrome, different types of renal pathology, and a tendency toward spontaneous remission. The approach to the patients must be individualized and determined on the basis of laboratory results and renal biopsy studies. Most physicians agree that the vast majority of patients can be carried successfully through pregnancy under careful observation by both obstetrician and internist. In certain patients, however, with moderate or severe hypertension, moderate to severe impairment of renal function, and demonstration of persistent (chronic) glomerulonephritis, termination of pregnancy may be advisable.

Nephrotic Syndrome in Association with Varicella

Spontaneously reversible nephrotic syndrome has been reported rarely in varicella infection (chicken pox); for instance, nephrotic syndrome occurred in three patients in a collected series of 2534 patients with chicken pox. Renal biopsies studied by LM had revealed diffuse proliferative change alone, or accompanied by thickening of the peripheral capillary loops. EM or IFM of the renal biopsies was not done in any of the reported series. Follow-up renal biopsy study in a single patient in one series demonstrated marked decrease in proliferative and membranous changes. In this patient, nephrotic syndrome completely disappeared, and at the time of second biopsy the urine was free of protein. With such rare reports, it is difficult to evaluate the relationships between varicella infection and renal disease. Nevertheless, the completely reversible nephrotic syndrome accompanied by diffuse proliferative glomerulonephritis suggests an acute process, which in turn supports the notion that this nephrotic syndrome is initiated by varicella infection.

SUMMARY

A step-by-step approach to the patient with nephrotic syndrome is essential. The action should be different for each age group.

1. Table 7-10 provides some perspectives to facilitate the workup for the causes of nephrotic syndrome in individual patients.

Numbers 4 to 9 for adults also apply for the elderly. In addition, the allergic state resulting from bee stings, pollen, etc., may account for nephrotic syndrome in a very small percentage of patients in all age groups. Of all the conditions enumerated, only a few of them are amenable to specific treatment and are reversible. The responsive conditions may be broken down into three categories:

A. Definitive: idiopathic nephrotic syndrome (lipoid nephrosis), secondary syphilis, drug use, allergy.

B. Potentially definitive: nephrotic syndrome in lymphoma.

C. Controversial: idiopathic membranous GN, lupus membranous GN, tumor-related membranous GN.

Table 7-10
Perspectives to Facilitate Workup of the Nephrotic Syndrome

Age group	Order of frequency
Infant	1. Congenital nephrotic syndrome 2. Congenital syphilis
Children and adolescents	1. Idiopathic nephrotic syndrome (lipoid nephrosis) 2. Focal glomerular sclerosis 3. Mesangioproliferative (mesangiocapillary) glomerulonephritis 4. Idiopathic membranous glomerulonephritis 5. Lupus membranous glomerulonephritis 6. Drug-induced (tridione) 7. Unhealed pathological processes, e.g., poststreptococcal GN, hemolytic uremic syndrome 8. Others, e.g., hereditary nephritis, chronic pyelonephritis 9. Secondary syphilis
Adolescents	The frequency of the causes of nephrotic syndrome would be almost similar to that in children. However, the incidence of focal glomerular sclerosis and membranous GN, idiopathic or induced by systematic lupus erythematosus, may be slightly higher in adolescents than in children
Adults	1. Idiopathic membranous GN 2. Idiopathic nephrotic syndrome (lipoid nephrosis) 3. Focal glomerular sclerosis 4. Lupus membranous GN 5. Mesangioproliferative (mesangiocapillary) GN 6. Unhealed poststreptococcal glomerulonephritis 7. Secondary syphilis 8. Rapidly progressive GN 9. Diabetic glomerulosclerosis 10. Tumor-related lipoid nephrosis 11. Tumor-related membranous GN 12. Amyloidosis
Elderly	1. Idiopathic membranous GN 2. Membranous GN due to a variety of neoplastic diseases 3. Amyloidosis 4. Focal glomerular sclerosis

The sole purpose of presenting this scheme is to focus on the absolute value of tissue diagnosis in nephrotic syndrome. It must be emphasized that in no case of nephrotic syndrome may pathological diagnosis be substituted for any laboratory test. However, in childhood nephrotic syndrome, renal biopsy may be deferred until a 6-week therapeutic trial with corticosteroid is completed.

2. In all cases, histopathological studies should be supplemented by adjuvant diagnostic tests to establish meaningful clinicopathological diagnosis.

3. Renal biopsy should not be avoided because of impaired renal function. This is often due to a contracted intravascular volume and often reverses following successful treatment. It is more than likely that nephrotic syndrome in long-

standing diabetes mellitus is due to diabetic glomerulosclerosis. This would be almost certain if the patient also manifests diabetic retinopathy and peripheral neuropathy. It should be noted, however, that membranous glomerulonephritis is observed in a variable percentage of patients with diabetic glomerulosclerosis, but the contribution of each of the two disparate pathological entities toward nephrotic syndrome is not yet established.

4. Attempts must be made to stage membranous GN because rational therapy can be advocated on the basis of staging.

5. Electron microscopy and/or immunofluorescence microscopy must be used to distinguish between lipoid nephrosis and stage 1 membranous GN. Both conditions may appear normal by LM.

6. All reversible causes of nephrotic syndrome, such as secondary syphilis or drugs, must be identified and promptly treated by penicillin or drug withdrawal, respectively.

7. There is little justification in recommending renal biopsy study in diabetes mellitus, especially long-standing diabetes mellitus. Renal biopsy study, especially by using EM, is of some value when the prediabetic state is under consideration.

8. Treatment of the tumor by surgery, radiation, or chemotherapy is often followed by remission of nephrotic syndrome. Remission is far more common in lymphoma than in solid tumors. Paradoxically, the appearance of nephrotic syndrome for the first time after the age of 40 years should arouse suspicion of an occult tumor.

9. Nephrotic syndrome during pregnancy may demonstrate pathological lesions of frequency similar to those found in nonpregnant adults. The obstetrician and internist must work together to provide the most appropriate management to such patient.

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8 Pathology of the Kidney in Profiling Acute Renal Failure (Acute Uremia)

DEFINITION

Acute renal failure (ARF) is an altered physiological state resulting from rapid but generally reversible decline of the regulatory functions of both kidneys or of a solitary kidney. Acute uremia is a clinical syndrome manifested by sudden onset of oliguria or anuria (24-hr urine output of less than 400 or 100 ml, respectively) for more than four successive days coupled with elevated values of serum urea nitrogen and serum creatinine.

In order to evaluate the importance of renal tissue diagnosis in the clinical care of acute uremia, a brief statement of the background to the problem is deemed necessary. The approach to the problem should be systematic and is as follows: (1) establishment of the syndrome of acute uremia, (2) implementation of adequate management to reduce mortality, and (3) establishment of the etiology of acute renal failure to determine the rehabilitation of the patient.

STEPS FOR THE COMPLETE MEDICAL CARE OF ACUTE UREMIA

The following are suggested steps in the handling of cases of acute uremia:

1. Thorough history and complete physical examination.
2. Routine laboratory tests.
3. Implementation of therapy.
4. Radiographic studies.
5. Plan for renal biopsy study.
6. Percutaneous renal biopsy study.
7. Outline of future plan.

Since this chapter is concerned mainly with the physiopathology of renal tubular lesions, a scheme of semiquantitative analysis of renal tubular lesions by light microscopy (LM) is stated here. This scheme facilitates assessment of the severity of the renal tubular lesions, especially if electron microscopy (EM) facilities are not available or EM study cannot be done. This type of LM assessment should be useful to clinicians for implementation or continuation of therapy and in calculating the prognosis of the disease process. The acute tubular lesions (ATL) are graded in order of increasing severity:

0	No lesion
1+	Loss of luminal membrane or brush borders
2−	Swelling and vacuolation of the cells
3+	Necrosis of the cells and separation of the cells from the basement membrane
4+	Same as 3+ along with basement membrane necrosis

It would be appropriate to describe 3+ to 4+ lesions as acute tubular necrosis (ATN), although throughout this text the term acute tubular lesions or ATL is used. This is mainly because severe lesions or ATN are not as common as is thought, whereas mild lesions which are referred to as ATL are quite common and possibly account for most cases of acute renal failure. This analysis and description of the lesions are in agreement with that of Olsen (1967).

Onset of acute renal failure is heralded by oliguria or anuria, especially when preceded by the following events:

1. Any severe medical illness, e.g., septicemia, persistent vomiting, severe diarrhea, massive gastrointestinal hemorrhage.
2. Surgical procedures, especially on the aorta and gallbladder.
3. Obstetric accidents, e.g., concealed hemorrhage (abruptio placenta).
4. Profound loss of fluid in an otherwise normal individual, e.g., excessive perspiration while working in a hot, humid climate.

In the absence of these histories and if there is a complete lack of information from the patient, oliguria suggests one of three possibilities: acute renal failure, chronic renal failure, or acute renal failure superimposed on chronic renal failure or chronic renal disease.

The history, physical examination, and laboratory tests listed in Table 8-1 will aid in distinguishing between acute renal failure and chronic renal failure. The presenting features of acute renal failure superimposed on chronic renal failure or chronic renal disease would be similar to these in chronic renal failure. A patient history is presented later to illustrate this example. First, let us discuss the optimum time for renal biopsy and how this study is going to affect the management and course of acute renal failure. In order to determine the rationale of renal biopsy, it is essential to review the various causes of acute renal failure. From the standpoint of clinical practice, the causes may be divided into:

1. Completely reversible causes: (a) dehydration; (b) bilateral intraluminal ureteral obstruction by stone, uric acid sludge, sulfonamide crystals, or blood

clots (due to, for example, administration of excessive doses of anticoagulant drugs)

2. Potentially reversible causes: (a) acute renal tubular lesions; (b) acute diffuse proliferative glomerulonephritis (GN) (poststreptococcal glomerulonephritis, lupus glomerulonephritis); (c) acute interstitial nephritis

3. Generally irreversible causes: (a) cortical necrosis; (b) rapidly progressive glomerulonephritis; (c) thrombotic renal lesions, e.g., thrombotic thrombocytopenic purpura, hemolytic uremic syndrome, hyperacute or acute graft rejection, malignant hypertension

Thus, it appears that the vast majority of cases of acute renal failure are caused by renal parenchymal diseases. Of all the causes just enumerated, acute tubular necrosis is considered by most observers to be the most common cause of acute renal failure. This statement may not hold true for all ages, for the incidence of renal disease varies somewhat among different age groups (see Table 8-2). Dehydration and electrolyte imbalance were the causes of acute renal failure in 50% of 122 elderly subjects in Belfast, Northern Ireland (Kumar *et al.*, 1973). These authors have stated that most elderly people live in a hydropenic state because of inadequate fluid intake. Hypovolemia and hyponatremia may result from injudicious use of diuretics, or major surgery with inadequate replacement of fluids and electrolytes.

Table 8-1
Clinical and Laboratory Information Useful for Differentiating between Acute Renal Failure and Chronic Renal Failure

Criteria	Acute renal failure	Chronic renal failure
Onset	Sudden onset of oliguria	Often forgotten, constitutional symptoms predominate
Clinical background	Remarkable history of recent events	No history of recent events
Physical examination	Generally normal	Generally abnormal in advanced state, e.g., pallor, itching, edema
Blood pressure	Normal (majority)	Hypertension (70–80%)
Neurological findings	None	Often present (uremic neuropathy)
Hemoglobin and hematocrit	Normal or slightly low[a]	Always low (average hematocrit, 25%)
Serum calcium	Normal, low,[a] or high[b]	Almost always low (7–8 mg/100 ml)
Serum phosphate	Normal, occasionally low[b]	Almost always high
Serum total protein and albumin	Normal	Generally low
Serum uric acid	Normal or low[a]	Almost always high
Metabolic acidosis	Mild	Moderate to severe
Cardiomegaly	None or slight	Frequent and moderate
Renal size (KUB film)	Normal or large	Frequently small (except polycystic or hydronephrotic kidneys)

[a] Owing to hemodilution resulting from excessive administration of fluid.
[b] Owing to transient hyperparathyroidism.
Source: From Mandal (1976), by the kind permission of the editor of the *Journal of the Indian Medical Association.*

Controversy exists concerning the value of renal biopsy study in the management of ARF. Whereas some physicians believe that renal biopsy may show no more than acute tubular necrosis, others undertake this study at least to rule out glomerular diseases, vascular diseases, and acute interstitial diseases. Although much disagreement prevails over the value of renal biopsy in the early stage of ARF, the objection is compromised if the oliguric stage lasts for 3 weeks or longer. Acute injury to each component of the kidney can give rise to acute renal failure. Thus, ARF may occur in acute glomerular diseases, acute tubular lesions, acute interstitial nephritis, and acute vascular thrombosis (arterial or venous). The causes of acute renal failure have been classified differently by various investigators. The classification provided by Olsen (1976) appears to be comprehensive and easily understandable (Table 8-3). In addition to the causes of acute renal failure presented in Table 8-3, I would suggest that acute renal failure following shock, sepsis, hemolysis, and so on, can also reveal extensive tubular necroses (3+ to 4+ lesions).

ACUTE GLOMERULAR DISEASES

Acute renal failure due to acute glomerulonephritis was once thought to be rare, but it does not now appear to be so. Renal biopsy studies have clarified the misconception. It is now clear that acute glomerulonephritis is not an uncommon cause of acute renal failure. Oliguria is a concomitant feature of poststreptococcal

Table 8-2
Causes of Acute Renal Failure in Different Age Groups

Age group	Renal diseases (tentative order of frequency)
Children and adolescents	Dehydration (prerenal) Acute glomerulonephritis Acute tubular lesions
Adults	Acute tubular lesions Acute glomerulonephritis Dehydration (prerenal) Acute interstitial nephritis Bilateral obstructive uropathy Papillary necrosis
Elderly	Dehydration Bilateral obstructive uropathy Atherothrombotic disorder (cortical necrosis) Acute tubular lesions Acute interstitial nephritis Acute glomerulonephritis Papillary necrosis

glomerulonephritis, lupus proliferative glomerulonephritis, or glomerulonephritis of Henoch-Schonlein syndrome. In these glomerular diseases, oliguric renal failure poses no special problem, since the primary disease process is easily recognized (by serological studies, quantitative urinary protein, etc.) and the urinary output increases with the remission of the glomerular lesions, which may occur spontaneously or with treatment.

It is important, but not essential, to establish the diagnosis of suspected poststreptococcal or lupus GN by histopathological study. It is imperative, however, to obtain renal tissue from patients who present no clinical evidence to indicate these diagnoses. It is not uncommon to see rapidly progressive glomerulonephritis in patients who present with very vague symptoms. To exemplify this possibility, a case history (patient S.B.) has been presented in Chapter 6. Chronic renal disease was found along with acute tubular necrosis and crescentic glomerulonephritis in a patient who was admitted with anuria of 2 days' duration.

E.S., a 67-year-old white male, was transferred to the University of Oklahoma Hospital in a semicomatose state on October 7, 1977, from a community hospital where he was first admitted in the same state the day before. The history was obtained from his brother, who stated that the patient had increasing swelling of the legs for 3 months and severe lethargy for 5 days prior to hospital admission. He was noted to have bloody sputum 2 days prior to admission.

Admission physical examination. General examination revealed semicomatose, wasted appearance, acute respiratory distress with respiratory rate of 40/min, blood pressure of 140/80 mm Hg supine and sitting, bleeding gums, the presence of fresh and old blood in the mouth, and dry skin. On systemic examination, deviation of the trachea to the right was found; rales and rhonchi were heard on both sides of chest and in front and back; heart sounds were obscured; however, an S4 gallop and a grade II/VI systolic ejection murmur were audible. Abdominal examination was unremarkable. Examination of the extremities revealed 1+ pitting edema. Complete neurological examination could not be done; there was symmetric decrease of deep tendon reflexes bilaterally. Stool was negative for blood.

Laboratory studies and hospital course. On admission: Serum chemistry, urea nitrogen 206 mg/100ml, creatinine 16.8 mg/100ml, Na^+ 137 mEq/liter, K^+ 6.9 mEq/liter, CO_2 8 mEq/liter, phosphate 6.4 mg/100 ml, calcium 6.8 mg/100 ml, total protein 4.7 g/100 ml, albumin 2.1 g/100 ml, uric acid 10.1 mg/100 ml. A few drops of urine were obtained by catheterization of the urinary bladder, and the specimen showed 3+ protein, numerous hyaline casts, one RBC cast, 15 to 20 WBC, and 5 to 10 RBC/HPF; chest x-ray showed opacification of the whole left lung and apex and lower lobe of the right lung and deviation of the trachea to the right; ECG showed peaked and tall T wave suggestive of hyperkalemia.

Because of hyperkalemia (serum K^+ 6.9 mEq/liter), severe azotemia, severe acidosis, and poor general condition, the patient was treated with hemodialysis, which was initiated soon after admission. His subsequent course is illustrated in Fig. 8-1. He had to undergo multiple insertions of A-V shunts due to clotting of the shunts. Other emergency measures included intubation and initiation of respirator on October 8 and extubation on October 14. Other laboratory studies consisted of negative ASO, ANA, and rheumatoid factor titers, and a serum C3 of 120 mg/100 ml. After 10 days of hemodialysis, he became alert and the cardiorespiratory condition markedly improved. On October 20, a percutaneous renal biopsy was performed and the tissue was studied by LM, EM, and immunofluorescence microscopy (IFM). (The results of these renal pathological studies are described later.) After 14 days of hemodialysis, his urine output began to increase steadily. After 24

Table 8-3
Types of Parenchymatous Acute Renal Failure Classified According to the Most Important Histological Lesions

Group	Pathology	Clinical forms
Glomerular and/or vascular lesions	Glomerulonephritis	Acute poststreptococcal glomerulonephritis (anuric type)
		Rapidly progressive (crescentic) glomerulonephritis
	Arteriolonecrosis	Malignant hypertension with anuria
	Arteriolar and glomerular capillary thrombosis, cortical necrosis	Microangiopathic hemolytic anemia
		Diffuse intravascular coagulation (obstetrically, sepsis, etc.)
		Hyperacute allograft rejection
Without glomerular or vascular lesions	Extensive tubular necroses	Toxic: mercury, chromium, uranium, etc.
		Some cases of primary ischemic graft anuria
	Acute tubulointerstitial nephropathy (tubular dilatation, casts, slight interstitial cellular infiltration)	Acute renal failure following shock, sepsis, hemolysis, myolysis, etc.
		Some toxic conditions (barbiturate, etc.)
	Interstitial nephritis	Acute lymphocytic interstitial nephritis caused by methicillin, sepsis, etc.
		Acute allograft rejection
		Acute and chronic pyelonephritis
	Minimal lesions or normal structure	Some cases of lithium poisoning
		Acute renal failure complicating nephrotic syndrome

Source: From Olsen (1976), by the kind permission of the author.

days, he was off the hemodialysis, his urine output increased to 1 liter/day, and serum urea nitrogen and serum creatinine leveled off at 50 to 60 and 10 mg/100 ml, respectively (Fig. 8-1). On November 3, a urine culture grew *Proteus mirabilis* (colonies greater than 10^5 ml^{-1}) and he was treated with ampicillin. In addition, he demonstrated intermittent confusion and disorientation. Because of poor mental status and lack of support from his family, he was not considered for a chronic hemodialysis program. His latest follow-up in January 1978 revealed no further improvement in either general condition or renal function. He had a urine output of approximately 1 liter/day.

Renal Pathology

Light microscopy. A total of 12 glomeruli were found in the section; eight were hyalinized and four showed necrosis and collapse of capillaries. The latter four glomeruli were compressed by florid crescents (Figs. 8-2 and 8-3). There were few tubules observed in the section; some were intact, whereas others showed disruption of the epithelium and debris within the lumen. The interstitium demonstrated fibrosis and infiltration by neutrophil, lymphocytes, and plasma cells (Fig. 8-2). A large arteriole showed no discernible damage except vacuolation of the smooth muscle cells.

Electron microscopy. Glomerular necrosis and crescent formation were confirmed (Fig. 8-4). The tubules showed normal to highly abnormal appearances; most of the proximal and distal convoluted tubules showed necrotic changes (Figs. 8-5 to 8-7), but the loop of Henle and collecting tubules remained largely unaffected (Fig. 8-8). However, thrombus was demonstrated within Henle's loops although the tubules were not necrotic (Fig. 8-9). A few tubules revealed features of regeneration and others showed atrophic changes. The interstitium was conspicuous due to infiltration by lymphocytes, plasma cells, fibroblasts, and collagen fibers (Fig. 8-10). An arterial vessel was studied and found to have evidence of necrosis (Fig. 8-11).

Comments

The histopathological study demonstrates a chronic and acute pathological process. The findings of hyalinized glomeruli, atrophic tubules, and interstitial

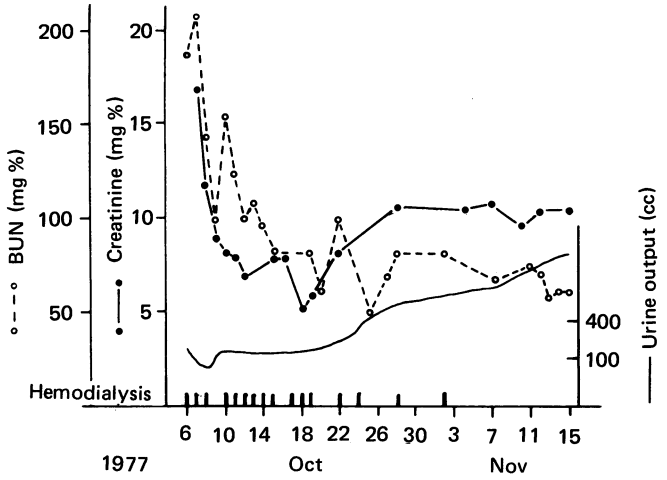

Fig. 8-1. Clinical course of patient E.S.

infiltration by neutrophils, lymphocytes, and plasma cells indicate chronic pyelonephritis. The acute pathological processes were characterized by crescentic glomerulonephritis and acute tubular necrosis. History of hemoptysis and appearance of dense opacities in the chest x-ray suggested hemorrhage in the lungs. The glomerular lesions comprising crescent formation, necrosis, and collapse of glomerular capillaries supported a diagnosis of rapidly progressive glomerulonephritis. No glomerulus was found in the portion of the tissue fixed for IFM. Lack of this study made it difficult to establish the diagnosis of rapidly progressive glomerulonephritis. Thus, this patient showed evidence of chronic renal disease, crescentic glomerulonephritis, and acute tubular necrosis.

Four important questions arise relative to this patient:

1. Did the renal failure develop acutely?
2. Did he have chronic renal failure?

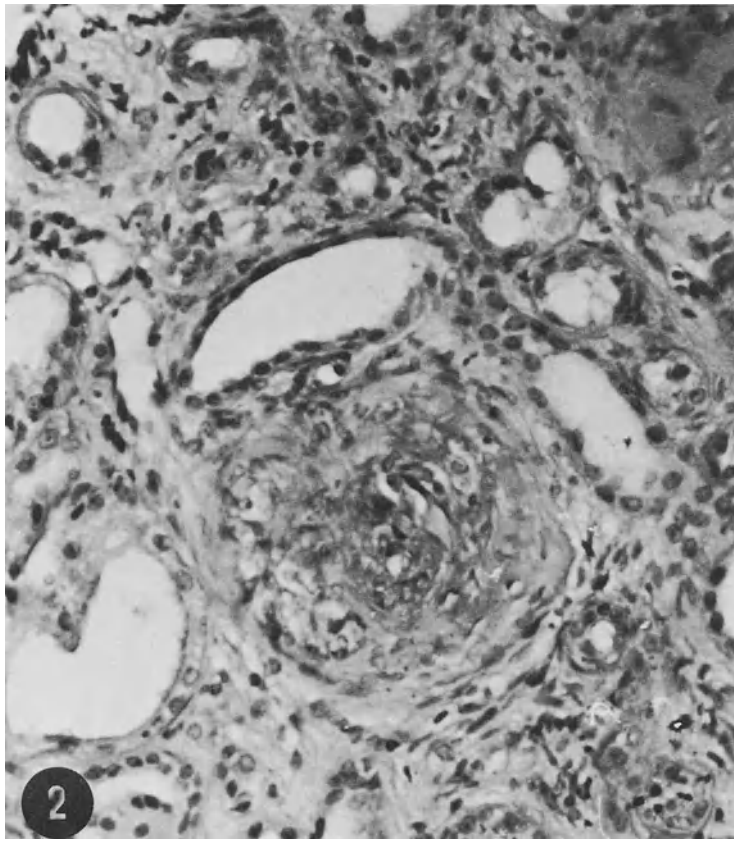

Fig. 8-2. A necrotic glomerulus with a fibroepithelial crescent, several dilated tubules, a few normal appearing tubules, mild cellular infiltration, and moderate fibrosis of the interstitium (H & E, ×120).

3. Was acute renal failure superimposed on chronic renal failure?
4. How much of each type of renal pathology contributed to the renal failure?

It is difficult to answer the first two questions because of inadequate information. However, acute renal failure superimposed on chronic renal failure is a distinct possibility. History of edema for 3 months prior to hospital admission suggests some kind of chronic renal disease. Histopathological study indicated chronic pyelonephritis which is supported further by pyuria and positive urine culture. Nephrotic syndrome is not uncommon in chronic pyelonephritis, nor is mild to moderately severe renal failure. A sharp deterioration of renal function might have been caused by crescentic glomerulonephritis. It is difficult to evaluate

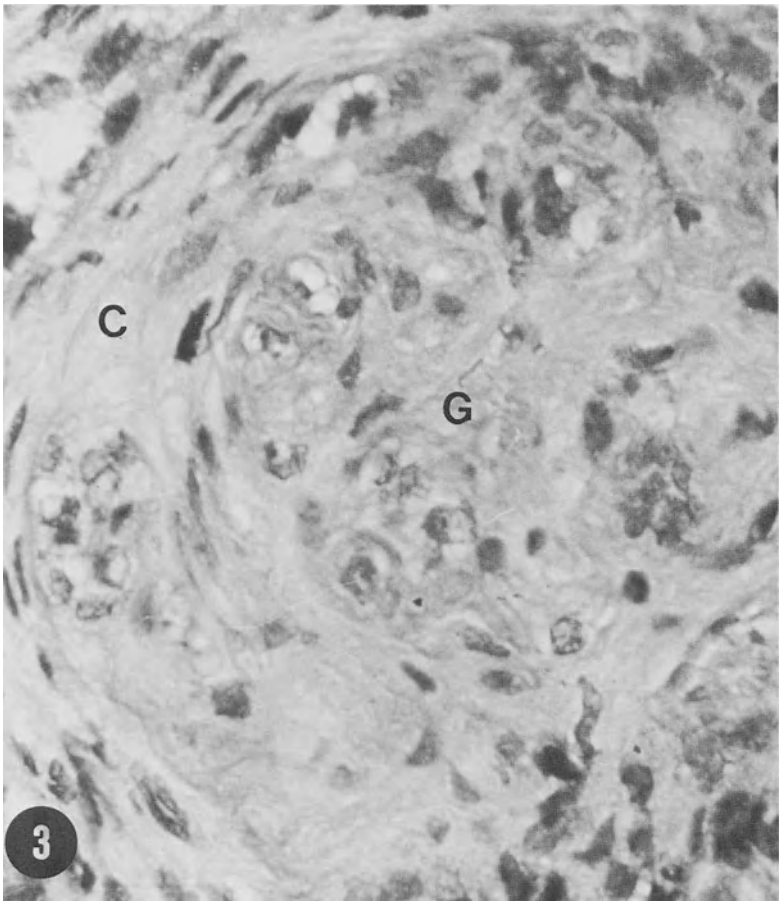

Fig. 8-3. Completely necrotic glomerulus (G) and the fibroepithelial crescent (C) are clearly shown (H & E, ×320).

whether or not necrotic and thrombotic changes seen in some of the tubules are part of crescentic glomerulonephritis or are indeed caused by ATN.

It is unknown whether the patient had stable chronic renal failure on which acute renal failure was superimposed, or whether the patient developed acute renal failure preceded by normal renal function. It is unlikely that a renal failure of this severity could be caused by observed tubular changes alone. It is more likely that the renal failure in this patient is the result of multiple renal parenchymal disease, as stated earlier.

Like idiopathic glomerulonephritis, acute glomerulonephritis induced by drug can present with acute renal failure. One such patient (C.S.) who was receiving penicillamine for rheumatoid arthritis suddenly developed epistaxis, thrombocytopenia, oliguria, and marked elevation of serum urea nitrogen. The clinical presentation and the histological studies of the renal biopsy have been elaborated in Chapter 6 (see Figs. 6-38 to 6-40).

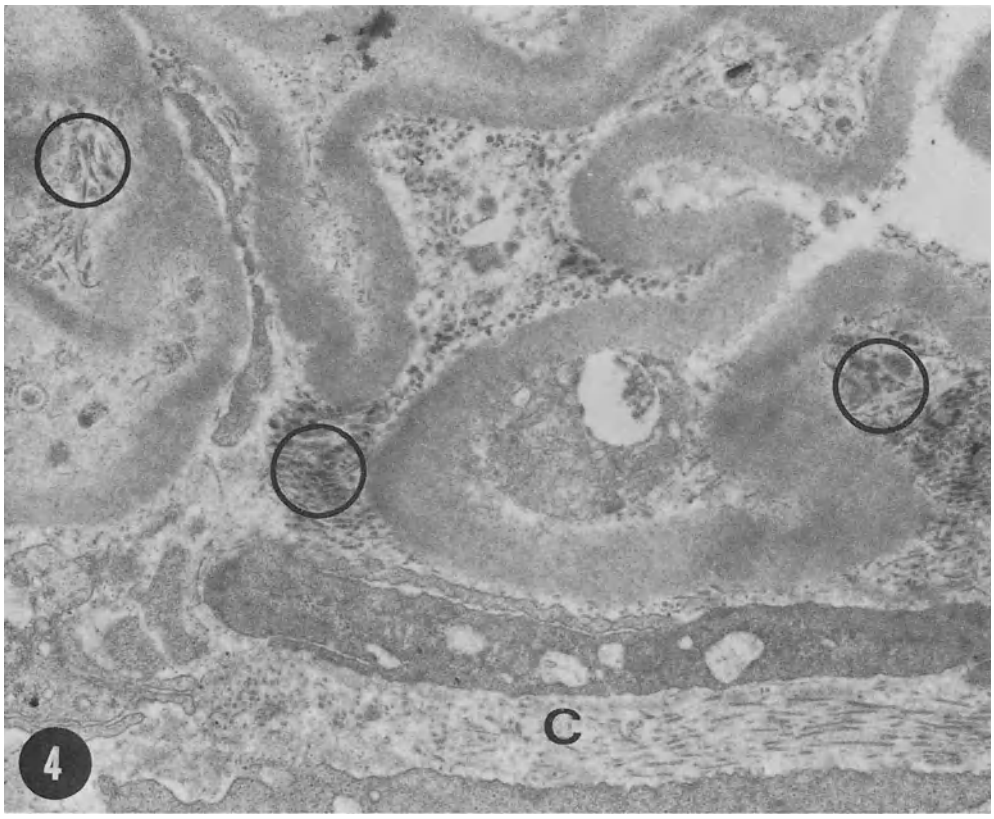

Fig. 8-4. Necrotic glomerular capillaries. Massive accumulation of collagen fibers (circles) within and between the necrotic capillaries. A part of the fibrotic crescent (C) is shown (UA + LC, ×20,000).

The cause of oliguria or anuria in acute forms of glomerulonephritis is not clearly established. Most investigators believe that oliguria is due to impediment of glomerular filtration caused by the occlusion or compression of the glomerular capillaries. In endocapillary proliferative GN, the glomerular capillaries are found

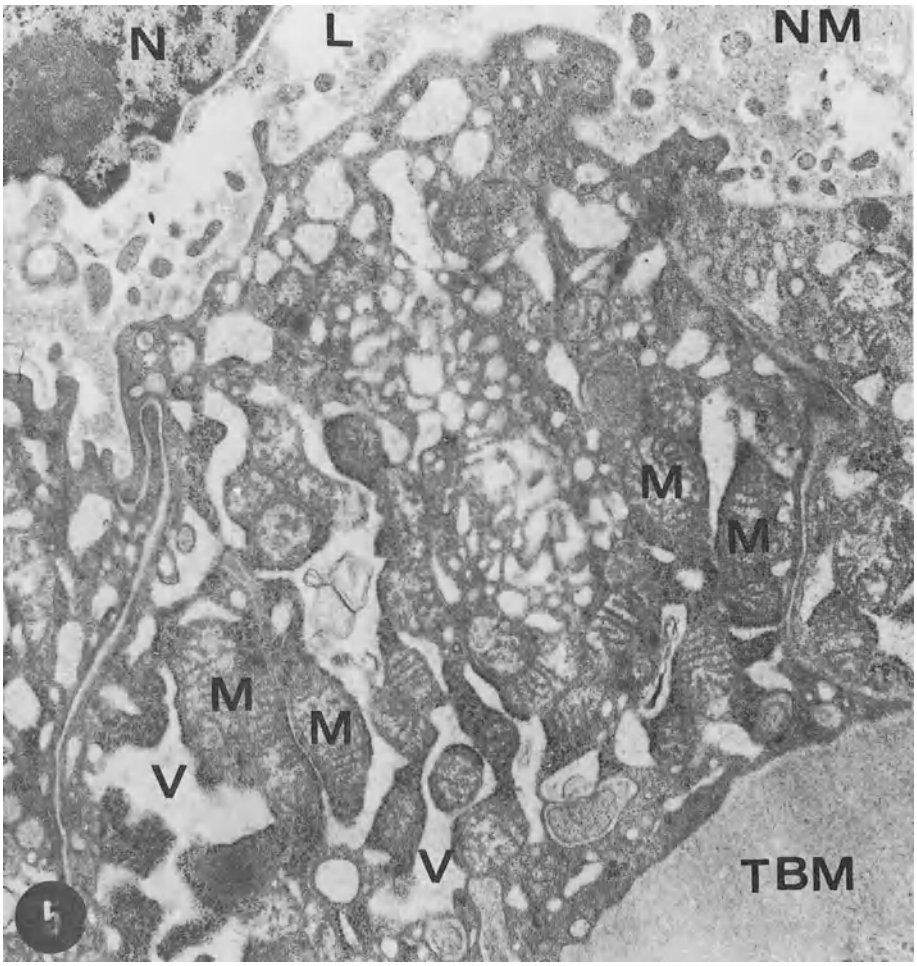

Fig. 8-5. A necrotic tubule is shown. The structures are so distorted that it is difficult to distinguish the anatomy of this tubule. Plasma membrane infoldings are not found at the basal part and are occasionally present between the cells. Nucleus (N) is necrotic and is located at the luminal part (L) of the tubule; mitochondria (M) are fused and associated with disruption of membranes and cristae and appearance of vacuoles. Many clear spaces or vacuoles (V) have replaced the cellular constituents. The basement membrane (TBM) is wide and structureless. Note necrotic material (NM) in the lumen (UA + LC, ×19,000).

to be occluded by proliferated endothelial cells and PMN exudation (see Fig. 6-1). This type of capillary occlusion may be the cause of oliguria, especially because urinary output increases and renal function tests normalize with the disappearance of proliferative and exudative changes. Serial renal biopsy studies in some of these cases have confirmed normal renal morphology. However, sequential histopathological studies have not been performed in enough cases to document that improvement in renal function is commensurate with the regression of the inflammatory process and opening of the lumina of the glomerular capillaries. In extracapillary proliferative glomerulonephritis, the glomerular compression resulting from florid crescent accompanied by collapse and necrosis of the glomerular capillaries may account for a marked decrease in glomerular filtration rate.

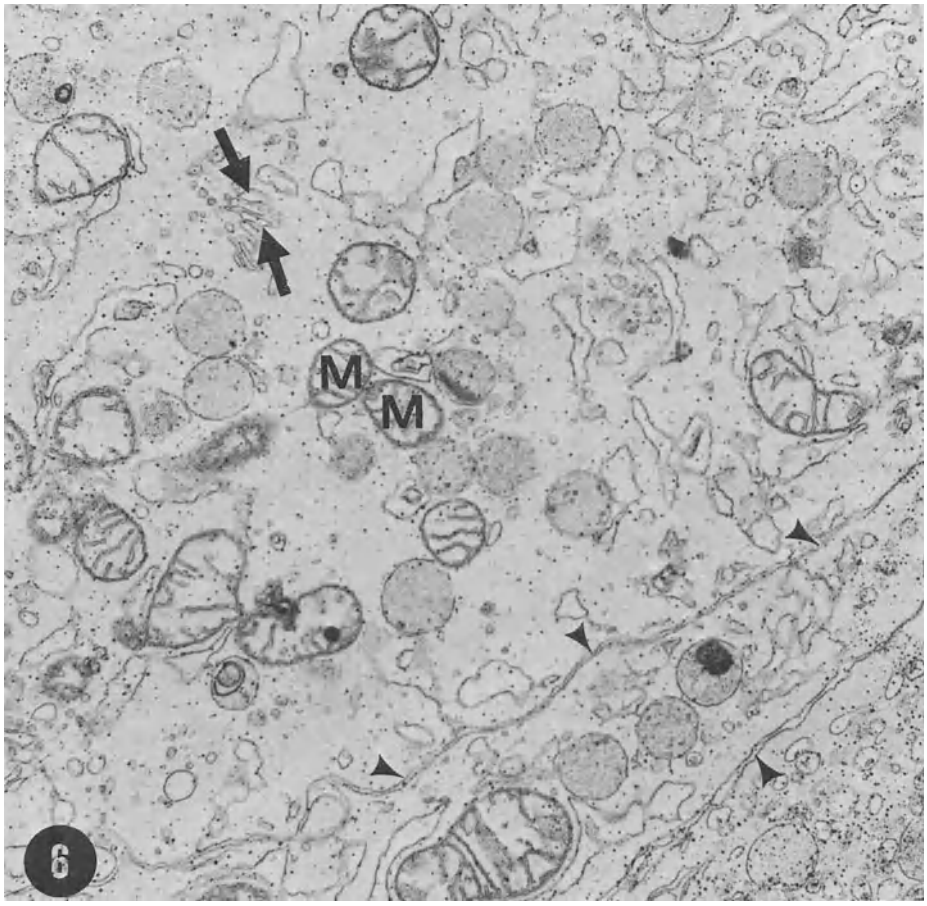

Fig. 8-6. Detailed definition of the necrotic tubule. Occasional cellular junctions can be discerned (arrowheads). Most of the cells are necrotic and edematous. There are a few intact mitochondria (M), but most mitochondria are undergoing dissolution. Remnants of Golgi complexes (opposing arrows) are shown (PAMS, ×18,000).

It is argued that oliguria is present in all cases of "glomerulonephritis"; however, it cannot be explained on the basis of glomerular changes alone. It was proposed that tubulointerstitial changes might contribute significantly to renal failure in glomerular diseases—in particular, in extracapillary proliferative GN. In our own experience, we have found severe interstitial changes characterized by edema and fibrosis associated with the presence of a few normal tubules in two patients with rapidly progressive GN. Some investigators made semiquantitative analyses of the tubulointerstitial changes and related them to serum creatinine or creatinine clearance in different types of glomerulonephritis. They

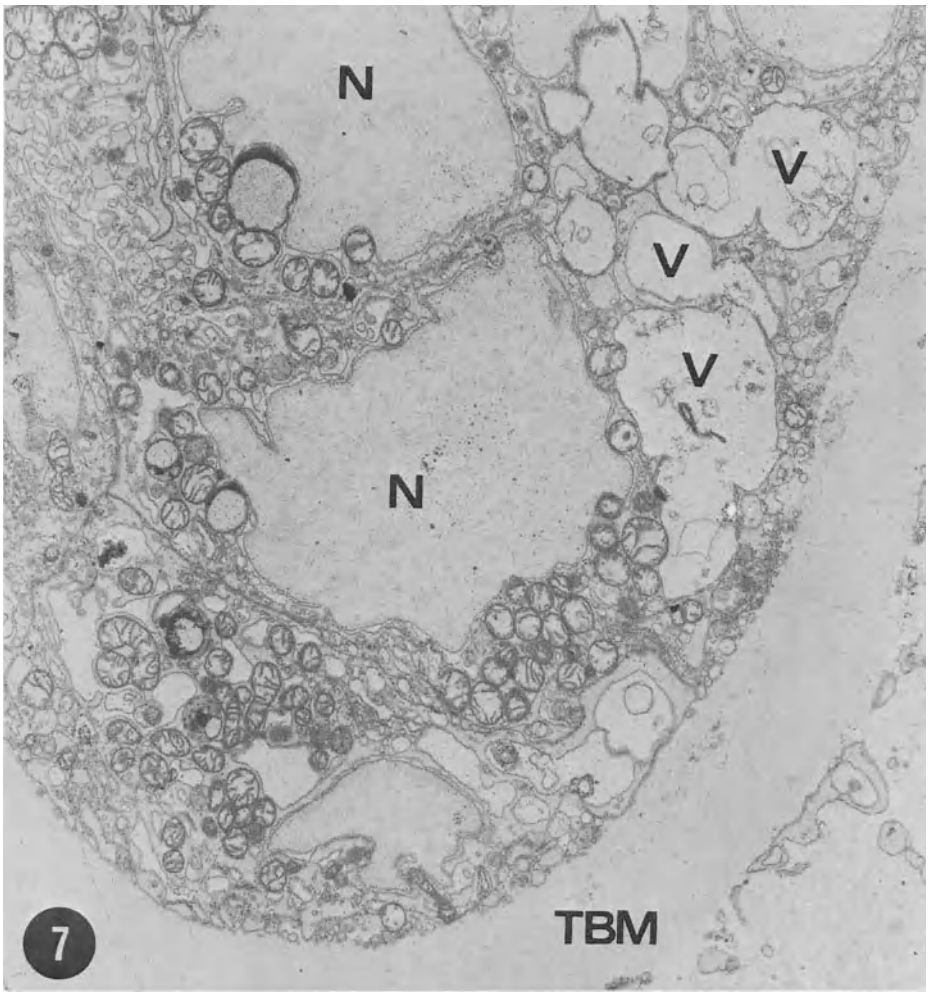

Fig. 8-7. Partial necrosis of a distal tubule. Note wide and edematous basement membrane (TBM), the absence of basal infoldings, and the presence of many vacuoles (V) in lieu of cellular constituents. Nuclei (N) reveal no discernible change (PAMS, ×9000).

concluded from their observations that creatinine clearances correlated better with tubulointerstitial changes than with the glomerular changes. This observation is reinforced by recent morphometric measurements made by Bohle and associates (1976). They showed that tubular changes similar to those seen in renal failure following shock, hemolysis, etc., could also be demonstrated in some cases of oliguric glomerulonephritis.

ACUTE RENAL TUBULAR LESIONS

The causes of acute renal tubular lesions may be broadly classified into so-called ischemic acute tubular necrosis and nephrotoxic acute tubular necrosis.

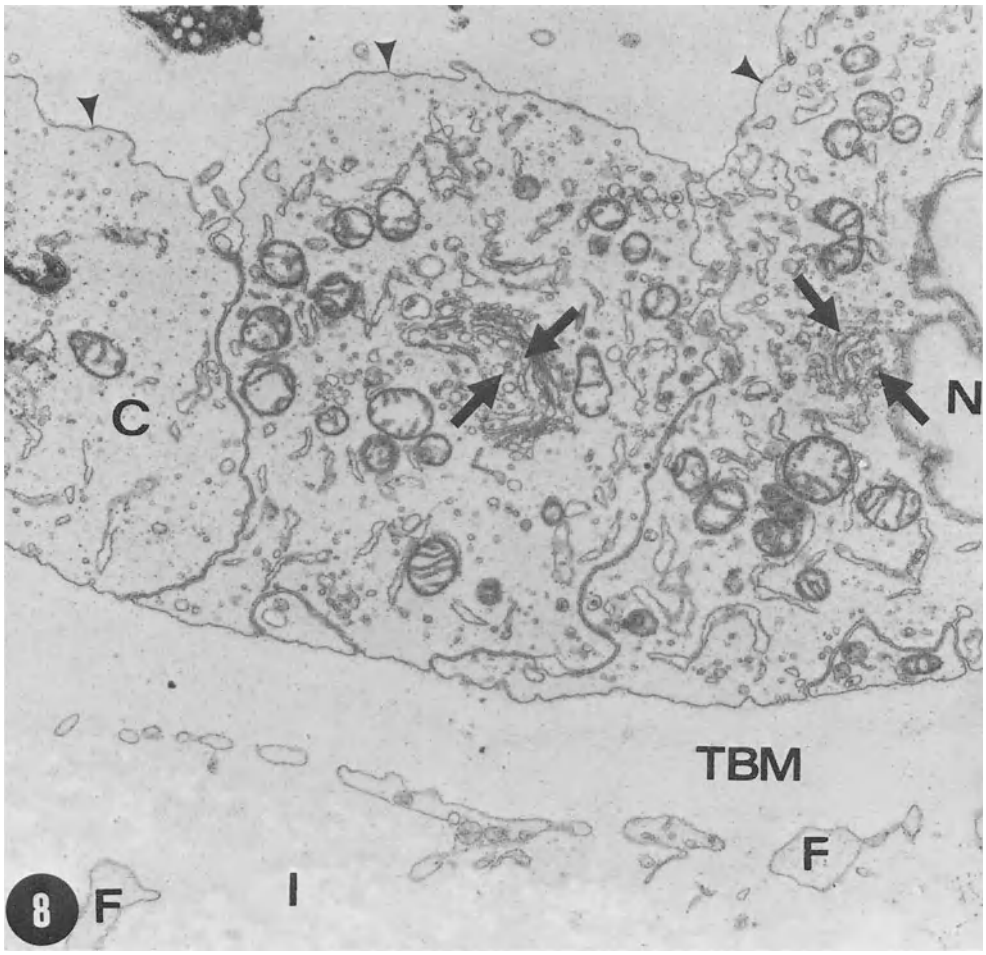

Fig. 8-8. Essentially normal collecting tubule. Whereas some tubular cells are rich in cell organelles, e.g., prominent Golgi complex (opposing arrows), other cells (C) have fewer cell organelles. Note intact luminal membrane of the cells (arrowheads). Basement membrane (TBM), wide interstitium (I), and fibroblastic processes (F) in the interstitium are seen (PAMS, ×14,000).

The common causes of so-called ischemic acute tubular necrosis are (1) surgical trauma, (2) shock and profound hypotension, (3) gram-negative septicemia, (4) crush syndrome or massive trauma, (5) myoglobinemia and myoglobinuria caused by acute myopathy, etc., (6) hemoglobinemia and hemoglobulinuria caused by mismatched blood transfusion, and (7) excessive hemmorrhage, e.g., gastrointestinal hemorrhage, obstetric hemorrhage. Severe (3+ to 4 +) acute renal tubular lesions or acute tubular necrosis can be divided further into uncomplicated ATN and complicated ATN. The complicated ATN includes the presence of thrombotic features associated with ATN, and it is caused by (1) severe shock, (2) massive trauma, (3) placental abruption, (4) gram-negative septicemia, and (5) postpartum acute renal failure. There are excellent experimental models of acute renal failure which resemble complicated ATN in humans; these are glycerol hemoglobinuric

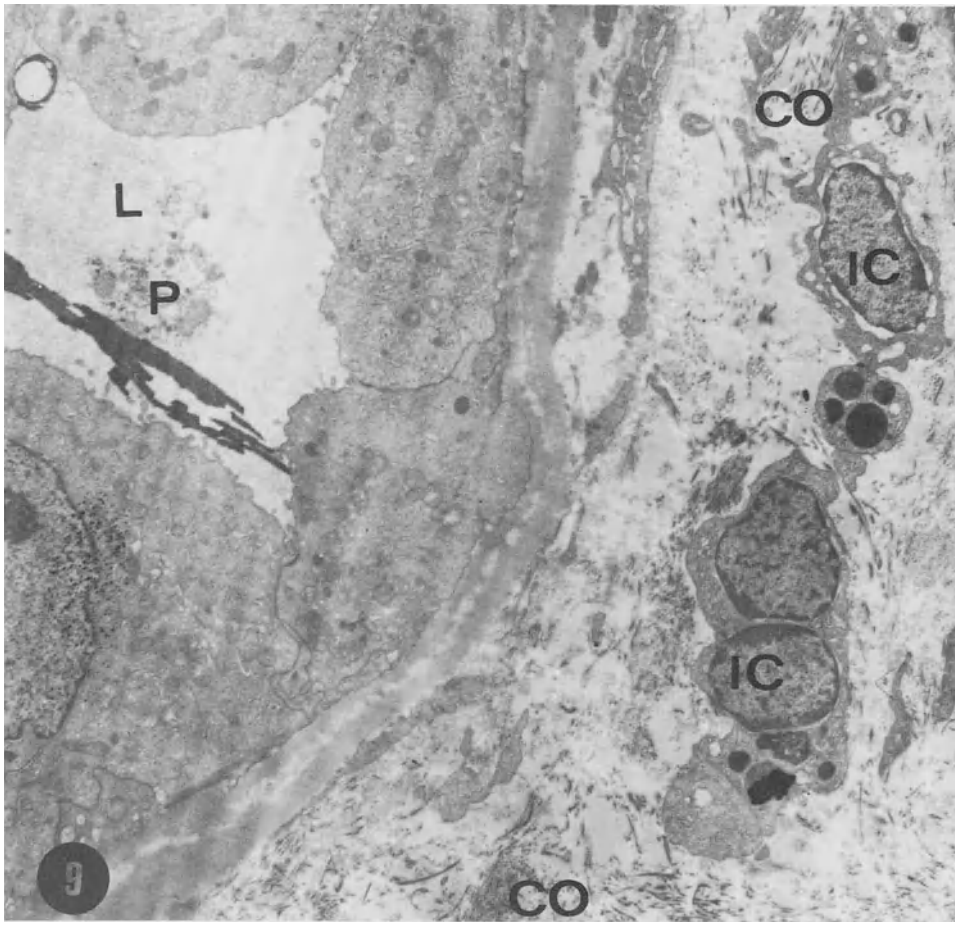

Fig. 8-9. A thrombus in the lumen (L) of an intact loop of Henle. The thrombus is composed of a degenerated platelet (P) and fibrin fibers. The interstitium is conspicuous due to the presence of many interstitial cells (IC) and collagen fibers (CO)(UA + LC, ×7500).

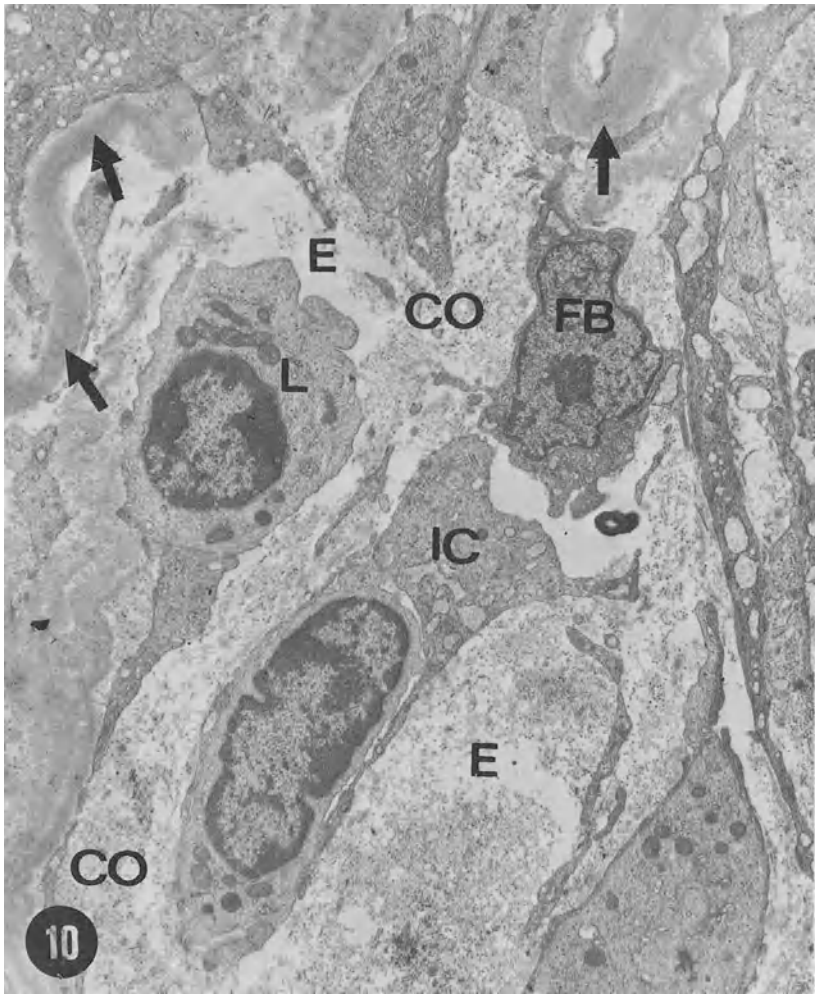

Fig. 8-10. A conspicuous interstitium due to the presence of numerous infiltrating cells. Lymphocytes (L), fibroblasts (FB), interstitial cell (IC), collagen fibers (CO), and edema (E) are shown. Note electron-dense basement membrane of the tubules (arrows) (UA + LC, ×8000).

nephrosis and epinephrine-induced acute renal tubular lesions. Stressful situations in the human, e.g., prolonged surgery, burns, obstetrical accidents, excessive trauma, or shock, can cause the outpouring of excessive amount of epinephrine and produce pathophysiological events consistent with ATN.

Two patients are described who developed complicated acute tubular necrosis following obstetrical intervention in one and gastric surgery in the other.*

* Studied by the author during a research fellowship with Dr. Robert C. Muehrcke, West Suburban Hospital, Oak Park, Illinois, and University of Illinois, Chicago, Illinois.

Patient #1, G.F., a 29-year-old Mexican female, was admitted to West Suburban Hospital, Oak Park, Illinois, for investigation and management of acute renal failure. She had been transferred from another hospital where dilatation and curettage of the uterus were done for an elective abortion. She became febrile on the second day after uterine intervention and developed oliguria on the fifth day. Admission physical examination was normal except for a termperature of 102 to 103°F. Urinalysis revealed 20 to 30 RBC/HPF; serum urea nitrogen and serum creatinine were 103 and 10.5 mg/100 ml, respectively. Blood cultures grew *E. coli* that was sensitive to ampicillin. An A-V shunt was inserted and she was dialyzed by hemodialysis beginning on the seventh postoperative day. She received ampicillin for infection. After three consecutive hemodialyses, a percutaneous

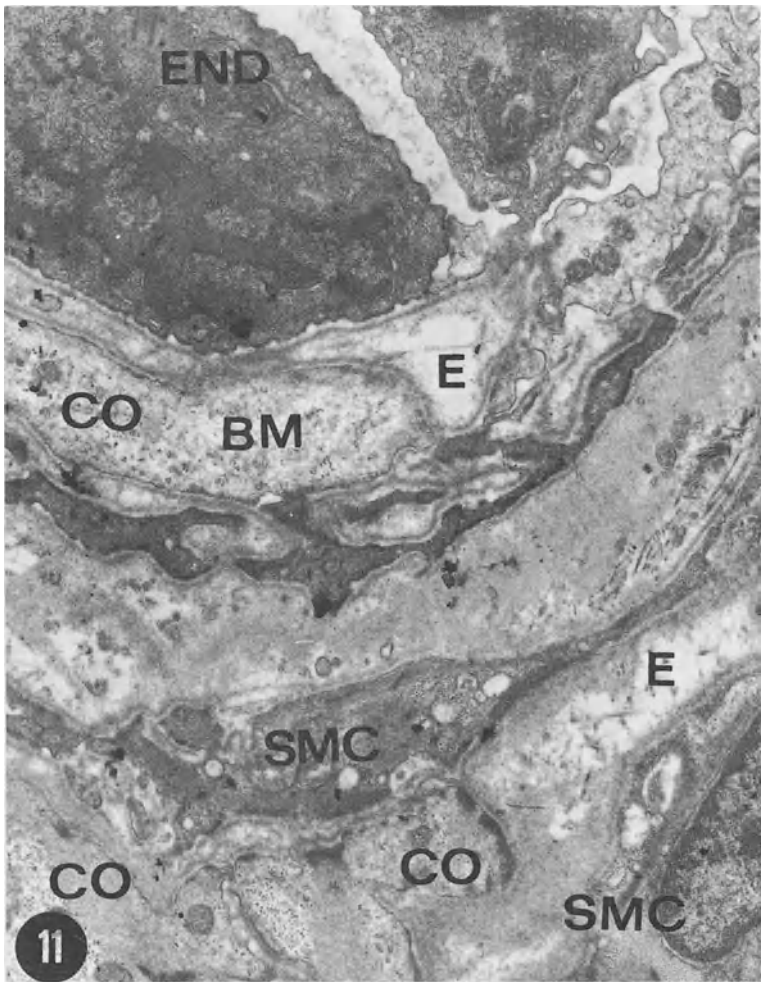

Fig. 8-11. In this arteriole, an intact endothelial cell (END) and infiltration of the basement membrane (BM) of the endothelial cell and smooth muscle cells (SMC) by collagen fibers (CO) can be seen. The electron-lucent areas within the BM represent edema (E). Most of the SMC are atrophic or necrotic (UA + LC, ×16,000).

renal biopsy was done and the renal tissue was studied by LM and EM. Light microscopy showed 3+ to 4+ tubular lesions and moderate to marked interstitial edema (Figs. 8-12 and 8-13). Electron microscopy confirmed LM findings and, in addition, demonstrated thrombi in the tubular lumina (Fig. 8-14). The EM study also showed regeneration of some tubules (Fig. 8-15), intact Henle's loops and collecting tubules, and mild glomerular changes. After daily hemodialysis for six consecutive days, her urine output increased to 4 liters, and blood urea nitrogen dropped to 65 mg/100 ml. Her urine output normalized in 2 weeks. The serum urea nitrogen and serum creatinine concentrations were steadily decreasing. After discharge from the hospital, she was lost to follow-up.

Patient #2, J.L., a 37-year-old Puerto Rican male, was transferred to the Nephrology

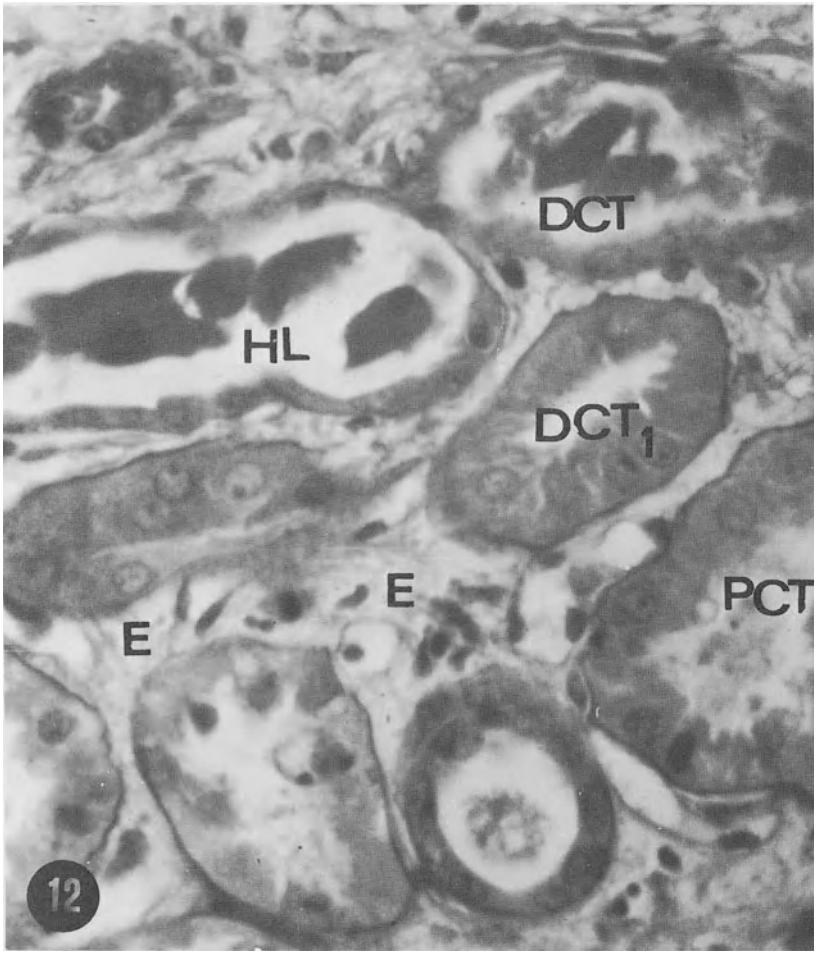

Fig. 8-12. Although most of the tubules display an abnormality, a few tubules are intact. A proximal convoluted tubule (PCT) demonstrates necrosis of the luminal aspect; Henle's loops (HL) contain necrotic material; several distal convoluted tubules (DCT) reveal necrotic changes, but a few distal convoluted tubules (DCT_1) appear normal. Tubules are widely separated by clear areas which represent interstitial edema (E) (PAS, ×200).

Service, West Suburban Hospital, Oak Park, Illinois, for acute renal failure which developed 2 weeks postsurgery for partial gastrectomy. On admission, he was very sick, stuporose, dehydrated, and febrile. The surgical wound was infected and grew *Pseudomonas* on culture, as did cultures of blood, urine, and sputum. His 24-hr urine output was 50 to 100 ml, serum creatinine 10 mg/100 ml, and serum urea nitrogen (SUN) 120 mg/100 ml. Both SUN and creatinine concentrations were rising faster than 20 and 1 mg/100 ml per day, respectively. He was considered to have hypercatabolic acute renal failure and

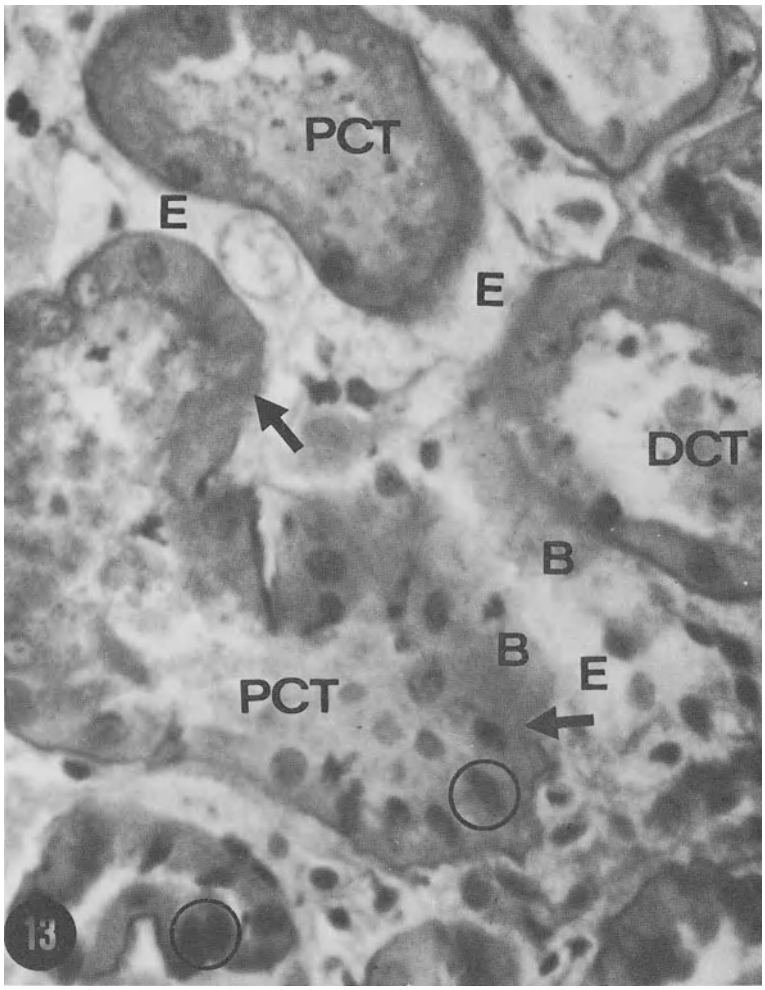

Fig. 8-13. Diffuse necrosis in the proximal convoluted tubules (PCT) and distal convoluted tubules (DCT) is clearly observed. The basement membranes of the tubules have thinned out or disappeared (arrows). Note bulgings (B) at the outer aspects of the tubules. These bulgings may represent passage of tubular contents into the interstitium. In the tubules a few normal nuclei are observed, but the remaining nuclei are poorly delineated. However, a few nuclei are dark (circles) which suggest regenerative changes. There is pronounced edema (E) of the interstitium (PAS, ×400).

was subjected to hemodialysis therapy. After a week of daily hemodialysis, he became alert. A percutaneous renal biopsy was done without any postbiopsy complication. The renal tissue was studied by LM and EM. In addition to hemodialysis, he received gentamincin parenterally, as well as locally in the abdominal wound. After 2 weeks of hemodialysis, his urine output began to increase rapidly, and by 3 weeks, his 24-hour urine output reached 6 liters. His SUN and serum creatinine began to fall rapidly. He made a complete clinical recovery and was discharged to go home. He was lost to follow-up.

The LM study revealed 4+ lesions in most of the tubules (ATN), and the EM study demonstrated necrotic and thrombotic changes. These findings were similar to those shown in the preceding patient (G.F.). The tubular basement membrane showed complete disruption and the interstitium exhibited a conspicuous edema (Fig. 8-16). Regeneration of some tubules was also observed (Fig. 8-17). Despite severe tubular lesions, glomerular changes were scarce and mild. Most glomeruli show no discernible change. The demonstrable glomerular changes consisted of swelling or vacuolation of endothelial cells, electron-dense glomerular basement membrane, hyperactive Bowman's epithelial cells, and

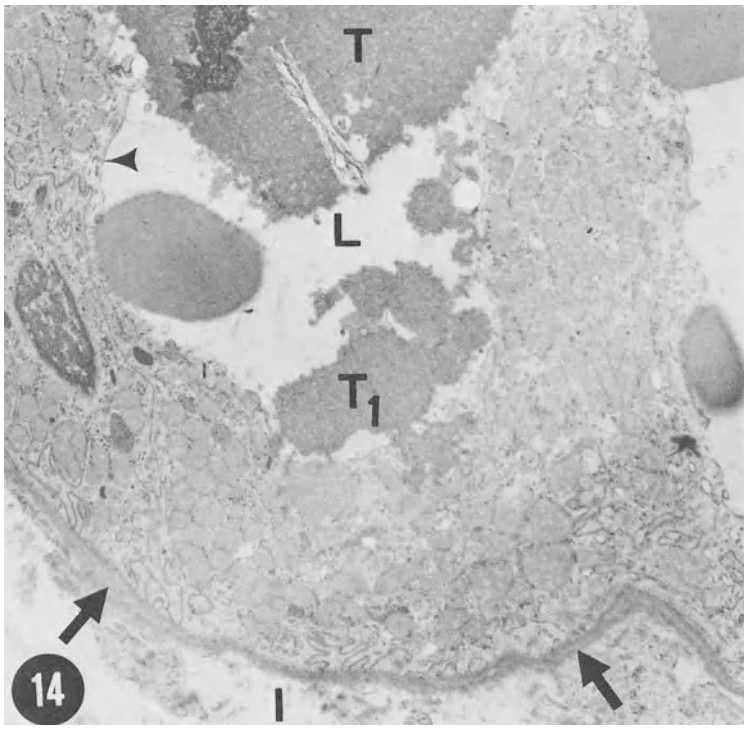

Fig. 8-14. A necrotic distal tubule shows thin and electron-dense basement membrane (arrows), conglomeration and poor definition of the mitochondria, inconspicuous plasma membrane infoldings, and a conspicuously large thrombus (T) within the lumen (L) of the tubule. Fragment of thrombus (T_1) is found to be attached to the apical surface of the tubule. Luminal membrane has disappeared in all but a small part of the tubule (arrowhead). Interstitium (I) is seen (UA + LC, ×6000).

fibrin and collagen fibers within the Bowman's space (Fig. 8-18). The arterial vessels were intact.

EXPERIMENTAL MODELS OF ACUTE RENAL FAILURE

Acute renal failure has been induced in rats, rabbits, dogs, and monkeys in order to study the mechanisms of oliguria and acute renal failure. The methods used to induce acute renal failure include intravenous infusion of epinephrine, unilateral direct intrarenal (arterial) infusion of epinephrine or norepinephrine, and intravenous infusions of a variety of nephrotoxic substances, e.g., hemoglobin, myoglobin, and uranyl nitrate. Of these models, the epinephrine-induced acute renal failure appears to be an appropriate model for the study of the physiopathology of acute renal failure in humans. The reasons for the application of this animal model to human study are manifold.

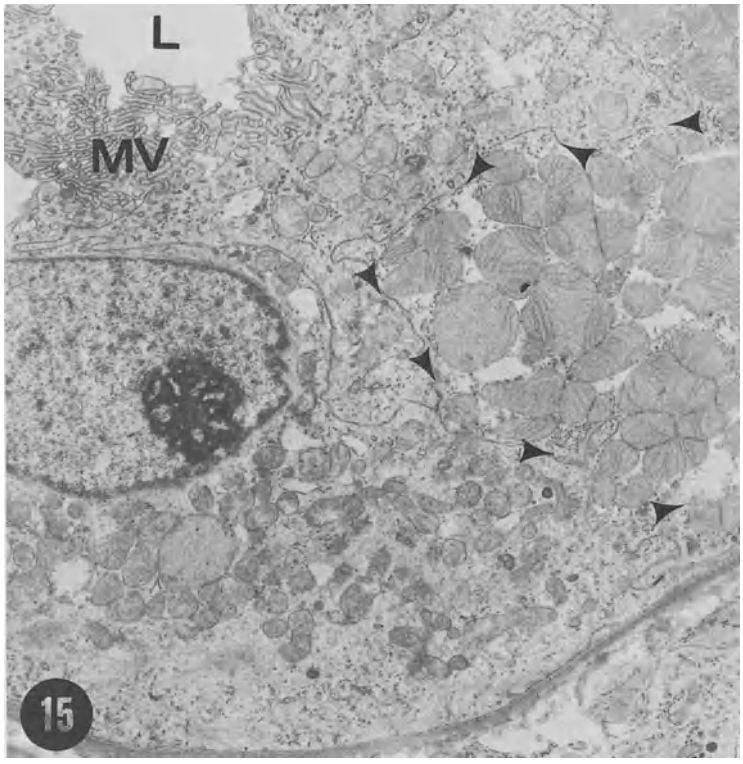

Fig. 8-15. This proximal tubule reveals necrotic as well as regenerative changes. The regenerative changes are characterized by a conspicuous nucleolus and a collection of intact mitochondria of variable size and shape in a cell (bounded by arrowheads). Note fragmented microvilli (MV) in the luminal aspect (L) of the tubule (UA + LC, ×6000).

Kidneys from dogs have been studied using light, electron, and immunofluorescence microscopy after continuous intravenous infusion of epinephrine at the rate of 4 μg/kg per min for 6 hr. The EM findings are illustrated in Fig. 8-19. The histopathological changes in these epinephrine-infused dogs resemble those in the two patients described in Figs. 8-12 to 8-18. A few dogs were observed up to 36 hr postexperiment for recovery of renal function, and none had urinary output.

Some authors have contended that stressful situations in the human, e.g., prolonged surgery, burns, obstetrical accidents, excessive trauma, or shock, can cause the release of excessive amounts of endogenous epinephrine and produce the physiological events and pathological states resembling those produced by *in vitro* infusions of epinephrine in dogs. However, the amount of epinephrine released endogenously under these stressful situations has not been assessed. It is also unknown whether the amount of endogenously released epinephrine is as much as the amount infused in dogs. It should be noted that smaller doses of epinephrine fail to produce ATL in dogs.

Oken (1976) has stated that increased sympathetic activity doubtlessly accompanies the initiating phase of vasomotor nephropathy (acute renal failure),

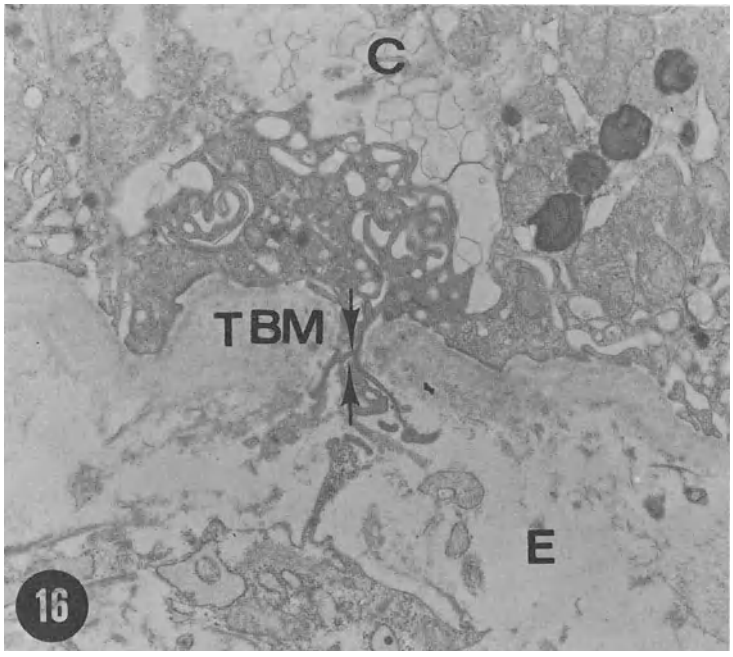

Fig. 8-16. A tubule demonstrates complete disruption of the basement membrane (TBM) (opposing arrows), passage of necrotic cells through the fragmented TBM, necrosis of the cells (C), and massive edema (E) in the interstitium (UA + LC, ×8200).

but he does not believe that such a state of heightened sympathetic activity persists through many days or weeks of renal failure.

Notwithstanding slight or no glomerular abnormalities, the mechanism of oliguria and acute uremia in acute renal tubular lesions has remained speculative. A variety of mechanisms have been proposed by numerous investigators, and include (1) decreased renal blood flow, (2) elevated renin-angiotensin level, (3) reduced renal prostaglandins, (4) vascular and glomerular thrombosis, (5) swelling of glomerular endothelial and epithelial cells, (6) tubular obstruction, and (7) tubular backdiffusion.

From a review of the literature and my own observations, I would like to provide a brief appraisal of supporting and contradictory views relative to the proposed mechanisms.

1. Renal ischemia. The decrease in renal blood flow or ischemia has been the most popular theory among all the proposed mechanisms. The popularity of this hypothesis has been compromised in recent years by contrary data emerging from several studies in humans and animals, two major items being (a) failure to

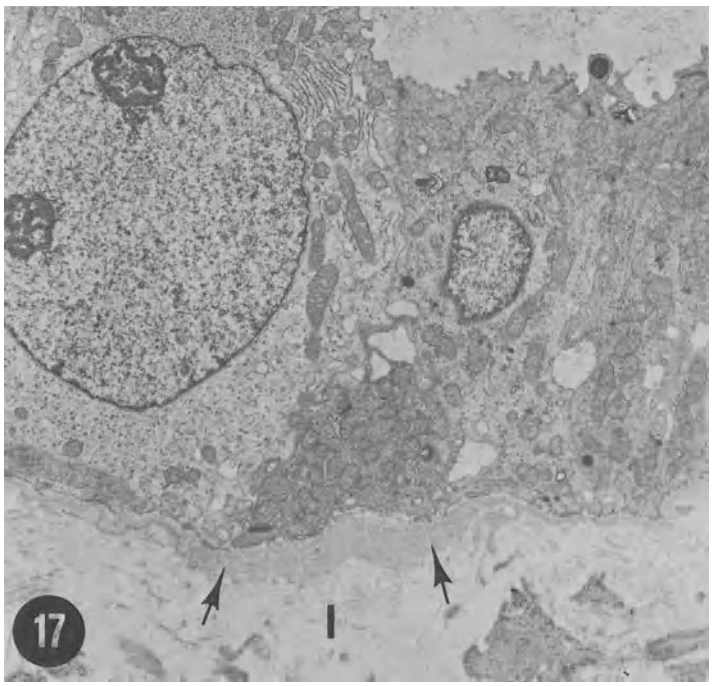

Fig. 8-17. Degenerative and regenerative cells of the loop of Henle. The degenerative cell has a small nucleus and the regenerative cell shows a large nucleus with two prominent nucleoli. Irregular and electron-dense tubular basement membrane (arrows) and interstitial (I) edema provide evidence of necrotic change in the tubule (UA + LC, ×6000).

reverse acute renal failure despite normalization of renal blood flow, and (b) findings of normal glomerular filtration dynamics in some models of acute renal failure.

2. The renin-angiotensin axis. Excessive production of renin-angiotensin has been implicated recently as the potential mediator of acute renal failure in the human. The supporting evidence for this proposal is the usual but not invariable elevation of renin and angiotensin concentratations throughout the period of sustained renal failure, decreasing toward normal shortly before the onset of renal recovery. Oken (1976) has stated that the facts argue strongly against the key role of circulating renin as the principal mediator of renal dysfunction. Reubi and Vorburger (1976) have suggested that increase in plasma renin activity may result from initial stress situations and bears no relationship to the development of anuria. These opposing statements are supported by the following observations:

a. Active and passive immunization to renin and use of competitive angiotensin antagonists or angiotensin-converting enzyme inhibitors do not prevent ARF.

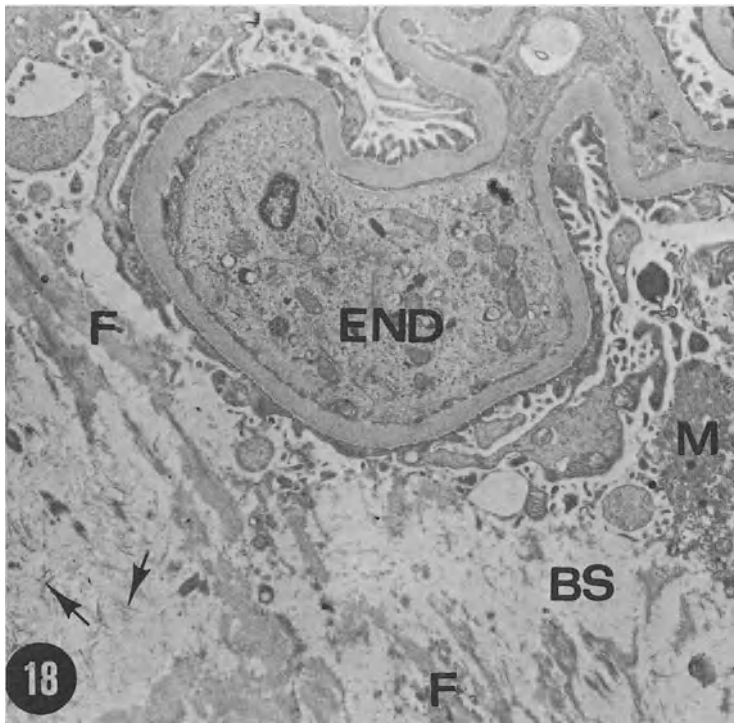

Fig. 8-18. This glomerular capillary reveals marked swelling of the endothelial cells (END). The presence of fibrin (F), collagen fibers (arrows), and necrotic masses (M) within the Bowman's space (BS) is evident (UA + LC, ×6000).

b. Tachyphylaxis to vasoconstrictor action of angiotensin II occurs rapidly in experimental animals.

c. Angiotensin II constricts both afferent and efferent arterioles, decreasing renal blood flow without changing the glomerular filtration rate (GFR).

d. Plasma renin activity may return to normal within 24 to 48 hr of insult, without reversal of ARF.

3. Renal prostaglandins. A relationship between renal prostaglandins and renal failure has been proposed. There are some studies which seem to support

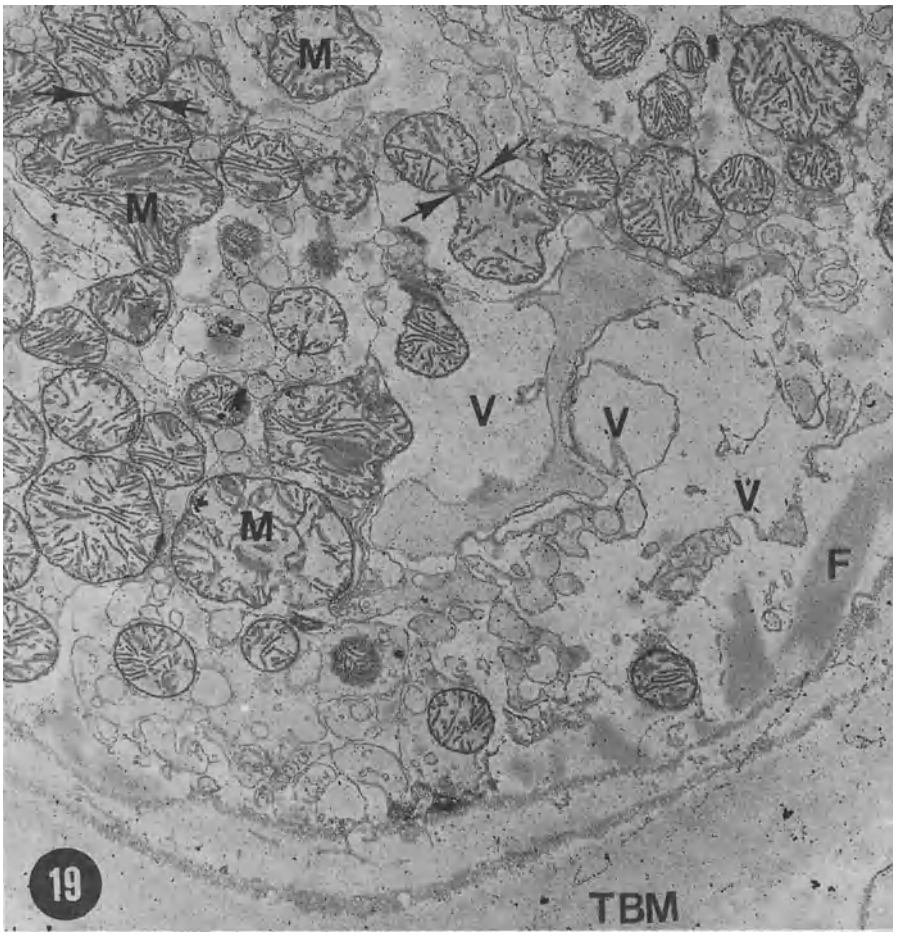

Fig. 8-19. A necrotic proximal convoluted tubule from a dog (after intravenous infusion of epinephrine 4 μg/kg per min for 6 hr) demonstrates replacement of much of the cellular constituents by vacuoles (V) and a decreased number of mitochondria. The mitochondria are of various sizes and shapes; there is fusion between them (opposing arrows), disrupted cristae, and the presence of homogeneous materials (M). The basement membrane of the tubule (TBM) is wide and structureless, suggesting necrosis. Note masses of fibrin (F) within the cells (PAMS, ×16,000).

the idea that reduction of renal prostaglandins may be an important pathogenetic factor in ARF. One of these studies reveals that use of indomethacin, a prostaglandin inhibitor, enhances glycerol-induced ARF in rats. In another study, Reubi and Vorburger (1976) observed marked increase of renal blood flow (up to 200%) but without diuresis after intrarenal infusion of prostaglandin E_1. There are conflicting reports on the levels (high or low) of renal prostaglandins in different experimental models of acute renal failure. Because of the contradictory observations, it is difficult to accept that alterations of prostaglandins play a key role in the pathogenesis of acute renal failure. However, it is reasonable to state that prostaglandin reduction may play a role in perpetuating the initial ischemic process in ARF.

4. Vascular and glomerular thrombosis. It is a well-established fact that thrombotic states are associated with acute renal failure. These processes may be broken down into two broad types: (a) thrombotic processes in which the coagulation profile is slightly or not altered, and (b) thrombotic processes in which the coagulation profile is markedly altered.

Thrombotic processes in which the coagulation profile is slightly or not altered include hemolytic uremic syndrome (HUS), thrombotic thrombocytopenic purpura (TTP), acute glomerulonephritides of diverse etiology, and toxemia of pregnancy. In these conditions, thrombi are found in the preglomerular arterioles and glomerular capillaries. Except for the appearance of low platelets in some cases, the clotting mechanism is largely unaltered. Alteration in coagulation factors in HUS has been reported by some investigators, but it has not been confirmed by others (for details, see page 125). It is unclear whether or not acute renal failure in these conditions is due to arteriologlomerular thrombosis alone or glomerulonephritis alone, or a combination of both.

Thrombotic processes associated with the complete disarray of the coagulation mechanism occurs in disseminated intravascular coagulation (DIC), which is often characterized by an abnormal coagulation profile, i.e., low platelet count, prothrombin level less than 60%, low or low normal plasma fibrinogen level, increased fibrin split products in serum and urine, and deformed or crenated red blood cells in the peripheral blood.

Conte and associates (1974) from Toulouse, France, have reported an autopsy study of 11 cases of DIC. Kidneys were involved in less than half of the cases (Table 8-4), and bilateral cortical necrosis was found in 50% of the cases with renal involvement. These authors also studied renal tissue obtained by percutaneous biopsies in the recovery phase of DIC in an additional nine patients. The results of the renal biopsy studies in these patients showed two normal, four with nonspecific tubular lesions, one with pregnancy nephropathy, and two with bilateral cortical necrosis.

Therefore, bilateral cortical necrosis was found in 4 of 20 cases, i.e. one-fifth of the total number of cases with DIC. Although all the cases did not exhibit renal cortical necrosis, all of them developed acute renal failure. This raises a question as to the cause of acute renal failure in the remaining 16 cases, which

did not show renal cortical necrosis. Nonspecific tubular lesions in four and normal histology (representing healed tubular lesions) in two did suggest acute tubular lesions as the cause of ARF in about one-third of the patients with DIC. These cases constitute extreme examples of DIC but there are cases of acute renal failure observed in clinical practice or at autopsy in whom DIC was not suspected or could not be documented. In many cases of ARF, renal biopsy is not done, or if it is done, the renal tissue is not commonly studied by EM or IFM. Since EM and IFM are considered to be the specific techniques used to confirm the presence of fibrin and fibrinogen, respectively, thrombotic renal lesions cannot be confirmed without these studies, and without them knowledge of the renal histopathological spectrum in ARF is vague.

It can be reasonably stated that ARF in some humans is associated with thrombotic renal lesions. Since these renal lesions of acute renal failure in the human resemble those induced by infusion of epinephrine in dogs, the factor of endogenously released catecholamines in the pathogenesis of renal lesions following stressful situations in the human is also a possibility. Since many of the stressful conditions, e.g., trauma, shock, burn, often are followed by DIC and acute renal failure, the idea that epinephrine plays a major role in the pathogenesis of ARF cannot be dismissed. This theory would be supported by the impressive studies of Whitaker and associates (1969) as well as McKay and associates (1969). By intravenous infusion of variable doses of epinephrine into rabbits and monkeys, these authors were able to produce DIC and necrotic organ lesions, including those in the kidneys, similar to those in the human.

The thrombotic renal lesions are not limited to DIC, but are observed in a variety of glomerulonephritides, malignant hypertension, toxemia of pregnancy, and TTP. Because all these conditions are accompanied by reversible or irreversible acute renal failure, there is probably a relationship between thrombosis and acute renal failure. The major question concerns differences in course, prognosis, and management between ARF associated with thrombotic renal lesions and that

Table 8-4
Distribution of Fibrin Thrombi

Group	Incidence	Location	Case ratio
I	100% of cases	Lungs	11/11
II	More than 50% of cases	Pancreas	7/11
		Spleen	6/10
		Adrenal gland	6/10
		Liver	6/11
		Gastrointestinal tract	5/9
III	Less than 50% of cases	Kidney	4/11
		Myocardium	3/10
		Brain	2/6

Source: From Conte *et al.* (1974), by the kind permission of Dr. J. Conte.

associated with nonthrombotic renal lesions. Thrombotic renal lesions seem to have a worse prognosis, and there is both concordance and disagreement among the reported series. Thus, Conte *et al.* (1974) did not find any difference between the cases of ARF with and without thrombotic processes. Andres-Ribes *et al.* (1975) found DIC in 27 of 86 cases studied. Although the mortality rate was higher in ARF with DIC than in ARF without DIC (59 versus 44%), the difference was not significant. Although there is no difference in immediate mortality, ARF with DIC more often develops permanent impairment of renal function. Heparin was tried with the hope of retarding the coagulation process and reversing ARF, but the results were generally disappointing.

5. Swelling of glomerular endothelial cells. The glomerular endothelial and epithelial cells appear essentially normal in most cases of uncomplicated acute renal tubular lesions. The glomerular changes, characterized by swelling of endothelial cells, necrosis of endothelial cells and basement membranes, proliferation of epithelial cells, and masses of necrotic and regenerative epithelial cells within Bowman's spaces, are commonly observed in association with complicated acute tubular necrosis (Fig. 8-18). But it is an unlikely possibility that these glomerular changes, with the exception of necrotic lesions, significantly alter glomerular filtration rate. Olsen (1967) studied 12 renal biopsies by EM from 10 patients with acute anuria, and in all but three biopsies, glomeruli showed slight, nonspecific damage to the cells. In the remaining three biopsies, swelling of endothelial and mesangial cells was noted. Olsen was not convinced that the fine structure of the glomerulus could explain the low glomerular filtration rate in acute anuria.

6. Tubular obstruction. Partial obstruction of the tubular lumina by necrotic epithelial cells and/or thrombi is seen in acute renal failure in humans and animals (Fig. 8-14). The tubular obstruction may lead to increased intratubular pressure proximally and to intrarenal hydronephrosis, which can further compromise glomerular filtration rate.

7. Tubular backdiffusion. It was suggested a long time ago that leakage of glomerular filtrate through the degenerative or necrotic tubular basement membranes may account for oliguria. This proposal has received good support from EM study of renal tissue in cases of acute renal failure. Electron microscopy has unequivocally demonstrated complete rupture of tubular basement membrane and passage of tubular contents through the disruption (Fig. 8-16). The tubular backdiffusion of the glomerular filtrate has been considered by numerous investigators to be the most probable cause of oliguria. There is evidence both in favor of and against this hypothesis, that in favor being as follows:

a. In most cases, normal appearance of the glomeruli by light and electron microscopy suggests a normal or near normal glomerular filtration rate. This is confirmed by micropuncture studies, which demonstrate near normal single-nephron GFR. This is supported further by injecting lissamine green intravenously and directly observing the exposed kidney by incident light. The dye appears in

the glomerulus and at the beginning of the proximal tubule but is not present in the terminal segments of the proximal tubule or in the distal tubule.

b. Necrosis and breaks of the tubular basement membrane have been convincingly demonstrated by electron microscopy (Fig. 8-16).

c. Radiological studies in cases of acute tubular necrosis reveal a dense nephrogram which may persist for hours or days. A positive nephrogram depends on the radioopaque material, usually diatrizoate, being filtered by the glomeruli and retained by the tubules. This has been confirmed by studies in rats after inducing acute tubular necrosis.

d. Radioactive inulin injected into the proximal tubule is no longer recoverable in the ipsilateral kidney.

e. Stein and Sorkin (1976) induced acute renal failure in dogs by injection of uranyl nitrate (5–10 mg/kg). Renal blood flow was measured and micropuncture studies were performed to evaluate the mechanism of the impaired renal function. The authors contend that in the nephrotoxic model, the maintenance of acute renal failure was caused both by leakage of filtrate across damaged tubular basement membrane and by a modest fall in GFR. Furthermore, a scanning electron microscopic study of the glycerol model of acute renal failure has suggested a tubular rather than a glomerular mechanism to explain continued renal failure in this model.

The evidence which argues against this hypothesis as the major cause of oliguria has slight or no practical application:

a. Necrosis and breaks of the tubular basement membrane are not a consistent feature in cases of ARF. This must be stated with great reservation, especially since renal biopsy study by EM is seldom done in ARF.

b. Saline overload can prevent mercuric chloride-induced acute renal failure despite severe tubular necrosis in rats.

c. Single-nephron GFR remains unchanged in some nephrons.

EVOLUTION OF HISTOLOGICAL REPAIR IN ACUTE TUBULAR NECROSIS

It is difficult to understand the sequence of histological repair of renal tubules because of lack of serial renal biopsy studies in the vast majority of cases of ARF. It is presumed that the histological repair depends on the severity of the initial lesion. The analysis of histological studies may vary according to the time interval between the onset of acute renal failure and the procurement of renal tissue, and the technique used to study renal tissue: LM alone or LM and EM.

By electron microscopy study, even during the oliguric or anuric stages, it is not uncommon to find some normal appearing tubules (Figs. 8-8, 8-15, and 8-17) along with severely damaged tubules. The normal-appearing tubules may have been spared the lesions or they may be regenerative tubules. The tubules

with uncomplicated necrotic lesions (3+ to 4+) undergo a rapid repair, and mild tubular lesions (1+ to 2+) may heal within days. This may explain the absence of acute tubular lesions of any severity if a renal biopsy is performed too late in the course of acute renal failure, especially of mild severity.

Muehrcke (1969) has stated by virtue of serial renal biopsy studies using EM that necrosis and regeneration of tubules go hand in hand. The repair of epithelial cells is characterized by mitosis, enlarged nucleus with prominent nucleoli or many nuclei, variability in size and shape of mitochondria, prominent cristae of mitochondria, and prominent Golgi complexes (Figs. 8-15 and 8-17). Many months are required for the complete healing of the epithelial cells of the cortical tubules. The evolution of the histological recovery of acute tubular necrosis is shown in Fig. 8-20. Serial renal biopsies were performed by Lewers and associates (1970) for undefined periods in 11 of 18 patients with histologically documented acute tubular necrosis. The renal tissues were studied by LM in all patients, by LM and IFM in a quarter of the patients, and by LM and EM in half of the patients. The abnormalities were mild and consisted of slight interstitial edema or fibrosis, debris in the tubular lumina, absence of tubular regeneration, and occasional obsolescent glomeruli. The IFM studies were unremarkable. The EM demonstrated increased intertubular collagen fibers and some thickening of the tubular basement membranes in two patients.

Even though the natural history of the healing of necrotic or sick tubules has not been extensively studied, the available data suggest that histological recovery

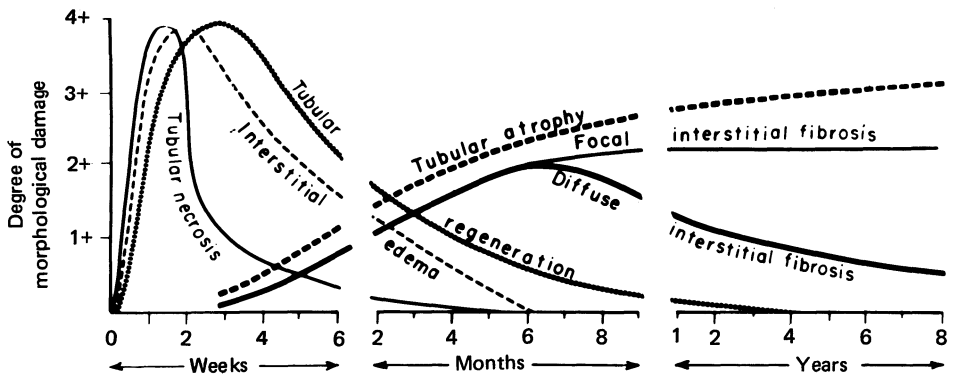

Fig. 8-20. This schematic diagram shows evolution of the histological recovery after acute tubular necrosis. Tubular necrosis, regeneration and atrophy, and interstitial edema and interstitial fibrosis were analyzed on a semiquantitative scale from 0 to 4+ in terms of weeks, months, and years. This diagram stresses the following: (1) Tubular regeneration is rapid and found simultaneously with necrosis. (2) Tubular atrophy and interstitial fibrosis appear within 2 to 3 weeks of the onset of acute renal failure. (3) Interstitial edema is more commonly associated with ATN (3+ to 4+ lesions) than with ATL (1+ to 2+ lesions). It tends to disappear as regeneration progresses. (4) Tubular atrophy is progressive in severe (3+ to 4+) tubular lesions. (5) Regeneration is complete in 6 months to 4 years. (6) There is apparently no correlation between the severity of tubular lesions and the degree of interstitial fibrosis. From Muehrcke (1969), by the kind permission of the author and C. V. Mosby Company.

of tubular lesions occurs in most cases. However, it is often accompanied by sequelae, i.e., variable degree of tubular atrophy and interstitial fibrosis (Fig. 8-20). We shall look at the effect of these morphological sequelae on the clinical and renal function status later.

ACUTE INTERSTITIAL NEPHRITIS

The simple meaning of this term is acute inflammation of the renal interstitium. Histologically, infiltration of the interstitium by neutrophil, eosinophil, or both has been observed. There is a growing interest in connecting this pathological state to acute oliguric renal failure, although realistically it is difficult to attribute acute renal failure to a histopathological state which is merely characterized by infiltration of the interstitium by inflammatory cells. Glomeruli and arterial vessels generally remain unaffected. The question of the involvement and the role of accompanying tubular lesions remains an important issue in this clinicopathological syndrome. The questions which may be asked with regard to this pathological entity are as follows:

1. How valid is the diagnosis of acute interstitial nephritis?
2. What is the relationship of the interstitial abnormalities to those of the neighboring structures, e.g., tubules, glomeruli, and arterial vessels?
3. Does acute interstitial nephritis alone, the lesions of the tubules alone, or both types of lesions together affect renal functions?
4. Is there direct proof that this pathological process is induced by drugs?

Muehrcke (1976) has stated that acute interstitial nephritis (a part of acute toxic nephropathy) is a by-product of the human's ever-increasing exposure to a vast array of chemicals resulting from the tremendous growth of industry and medicine. There is an ever-increasing suspicion of this possibility, especially because of our greater awareness of renal complications following excessive use of antibiotics in infectious processes.

Recognition of Acute Interstitial Nephritis

Acute interstitial nephritis (AIN) can be recognized by means of history, laboratory tests, renal biopsy study, and course of the disease.

History

History can be separated into background information and clinical presentations.

Historical background includes the following points: First, several decades ago, Kimmelstiel (1938) reported acute interstitial nephritis in association with hemolytic reactions following blood transfusions and in the hepatorenal syndrome. Second, acute pyogenic interstitial nephritis has been observed in asso-

ciation with urinary tract infection, septicemia, scarlet fever, brucellosis, and so on. Third, hypersensitivity acute interstitial nephritis, occurring mainly in association with pharmacological agents, has also been observed. There is great interest among clinicians and pathologists in undermining hypersensitivity acute interstitial nephritis. Many drugs, mostly antibiotics and sulfonamides, have been implicated as producing acute interstitial nephritis. These drugs are penicillin; semisynthetic penicillins, e.g., methicillin, ampicillin; penicillin analogues, e.g., penicillamine; sulfonamides; sulfonamide derivatives, e.g., furosemide; diphenylhydantoin (dilantin); cephalothin; allopurinol; azathioprine; and anticoagulants, e.g., phenindione. Of these, methicillin has received the most discredit. Since methicillin has been implicated as the causative agent for AIN by most observers, it may be convenient to use the term methicillin-induced acute interstitial nephritis. Because of the similarities in clinical presentations and histological findings between acute interstitial nephritis induced by methicillin and that caused by the other drugs just listed, the remaining information is given under the heading of methicillin-induced acute interstitial nephritis.

Methicillin-Induced Acute Interstitial Nephritis. Because of isolated case reports, it is difficult to estimate the exact incidence of renal lesions caused by methicillin or any of the other drugs. In three large reported series, when large doses of methicillin were used in staphylococcal septicemia, osteomyelitis, and staphylococcal endocarditis, the incidence of renal involvement varied from 15 to 17%. The renal involvement appears to depend more on the duration than dosage of methicillin administration. It is uncommon when the treatment lasts for 10 days or less, and it is most common if treatment exceeds 2 weeks. Thus, Nolan and Abernathy (1977) found highly abnormal urinalysis in 9 of 52 (17%) patients in whom methicillin treatment extended between 13 and 21 days. In contrast, abnormal urinalysis of similar severity was observed in patients receiving from as low as 4 g to as high as 12 g of methicillin daily. Olsen (1976) found severe interstitial infiltration with mononuclear cells in renal biopsies from patients who received prophylactic methicillin for cardiac surgery.

Clinical Presentations and Laboratory Tests

The clinical presentations include fever, skin rash, oliguria, and bloody urine. However, oliguria is more common than the other features. The results of the laboratory tests are as follows: Urinalysis varies from 1 + to 4+ protein and from a few to innumerable RBC and WBC. There is usually mild to moderate elevation of serum urea nitrogen and serum creatinine. Peripheral blood smear demonstrates elevated eosinophil count with or without leukocytosis.

Of all the clinical features and laboratory tests, positive urinalysis and azotemia (elevation of serum urea nitrogen) appear to be consistent features. The observation of these two features in patients receiving methicillin provokes suspicion of acute interstitial nephritis. Although azotemia is almost always found, progressive acute renal failure is uncommon.

There are few renal biopsy studies available among the many reports on methicillin- or drug-induced renal diseases. Although renal biopsies might have been performed by several investigators, few have studied the tissue using EM and IFM techniques.

The finding of severe interstitial infiltration with a relative sparing of glomeruli is the pathological hallmark of this disease. Eosinophilic infiltration and acute tubular changes appear to be characteristic features of this pathological process and may serve to distinguish it from chronic interstitial nephritis. Involvement of the tubules is easily recognized via EM. In the study of Ooi and associates (1975), both proximal and distal tubules were found to be affected, the distal more than the proximal. The EM findings were quite similar to those observed in acute tubular necrosis. Neutrophils and lymphocytes were found to infiltrate tubular epithelium and were considered diagnostic features of AIN.

Course

In most cases, azotemia is mild and rarely requires dialytic therapy. Confirmation of the diagnosis is provided by prompt recognition of the complication and withdrawal of the drug. Nolan and Abernathy (1977) observed normalization of urinary abnormalities and azotemia in all but one patient after withdrawal of methicillin. The normalization was fast in most cases. One patient required hemodialysis for 2 weeks. This finding is in agreement with that of Muehrcke (1969) and others.

Diagnosis of Acute Interstitial Nephritis

The diagnosis of acute interstitial nephritis can be made in most cases by circumstantial evidence. Circumstances include renal complications (hematuria, azotemia, and oliguria) appearing in patients treated with a potentially nephrotoxic drug. Other features include skin rash and peripheral eosinophilia, which are found in less than 50% of the cases. Even so, they suggest hypersensitivity reaction, but not necessarily renal disease. Renal biopsy is not always performed; if it is done, the mere histological appearance of interstitial infiltration is not enough evidence to establish a distinct pathological entity. This is difficult, especially in view of the fact that interstitial changes are almost always associated with variable degrees of tubular damage. Therefore, there remain several critical issues. First, can these clinicopathological abnormalities be recognized as an acute interstitial nephritis *per se*? Second, what is the relationship between the changes in the interstitium and the changes in neighboring structures, i.e., tubules, glomeruli, and arterial vessels. Although glomeruli and arterial vessels generally remain uninvolved, tubules almost always demonstrate definitive changes. Since light microscopy is not a good technique for the study of tubules, negative tubular findings by LM can be attributed to an inability to recognize

tubular changes, especially of mild or moderate severity (1+ to 2+ lesions). Electron microscopy study demonstrates definite changes similar to those in acute tubular necrosis. This has been described adequately by Ooi and associates (1975). It is not uncommon to find inflammatory cells in the interstitium which are associated with acute tubular necrosis, especially when renal tissue is studied by EM. Therefore, the argument is whether or not the entity of acute interstitial nephritis exists as such, or whether it is more appropriate to state that this entity be called acute tubulointerstitial nephritis.

Third, it is totally unknown which of the components, i.e., interstitial changes, tubular changes, or both, can be held responsible for the clinical syndrome of hematuria and azotemia (renal failure). There are plenty of valid experimental and clinical data to support acute tubular lesion, in contrast to a few or none in support of acute interstitial nephritis alone, as the cause of acute renal failure. Therefore, it is logical to assume that tubular changes are as important as interstitial changes for the clinical syndrome. Consequently, attempts should always be made to demonstrate or eliminate tubular changes.

Fourth, there is no direct evidence to support the prevalent notion that a drug, e.g., methicillin or penicillin, alone produces acute interstitial or tubulointerstitial nephritis. It must be remembered that the infectious process itself for which the antibiotic or antibiotics are administered, or the complicating shock, or a combination of both can also produce similar tubulointerstitial changes. This opinion is in accordance with that of Nolan and Abernathy (1977).

If we have to believe that acute interstitial nephritis is a distinct entity, the obvious question remains as to the mechanism of this pathological process. No definitive mechanism is known thus far. Immunofluorescence study of renal tissue was done rarely and the findings were either unimpressive or inconsistent. However, three pieces of evidence provide some insight into the mechanism of this disease: (1) Elevated serum IgE levels in a few patients have been reported by Ooi *et al.* (1975); this finding coupled with peripheral eosinophilia suggests that reaginic antibody complexes may be involved in the pathogenesis of the lesion. (2) Antitubular basement membrane (anti-TBM) antibodies were detected by indirect immunofluorescence in the serum of a patient who developed severe renal failure during treatment with methicillin. Renal histopathological study revealed severe infiltration of the interstitium by mononuclear cells. IgG, C3, and a methicillin antigen assumed to be dimethoxyphenylpenicilloyl were present in a linear pattern along the tubular basement membrane only. This observation apparently suggests that an immune mechanism may be involved in the pathogenesis of AIN. This is supported further by the immunofluorescence findings of the binding of penicilloyl hapten to kidney tissue along with IgG in a patient who had acute interstitial nephritis and developed acute renal failure following penicillin therapy. (3) The findings in experimental models of tubulointerstitial nephritis are similar to those in patients (for more information, see Chapter 10).

Regardless of the definition of the pathological process, the fact that the clinical syndrome regresses following withdrawal of the drug while other circum-

stances remain unchanged virtually proves that the drug or drugs play a significant role in this clinicopathological syndrome.

ACUTE RENAL FAILURE ASSOCIATED WITH LESIONS OF THE SMALL VESSELS OF THE KIDNEYS

Acute renal failure has been reported in a small number of cases of malignant hypertension. Progressive deterioration of renal function is most common in severe or malignant hypertension, whereas abrupt onset of renal failure is uncommon. Renal failure in hypertension has been attributed to arterial vascular changes, glomerular changes, or both by most observers. The role of tubules and interstitium was hardly, if ever, considered in the pathogenesis of renal failure in hypertension. Sevitt and associates (1971) reported tubular necrosis, which was thought to be a major cause of acute renal failure, in some of their patients with malignant hypertension. We have studied a patient with severe hypertension who demonstrated dramatic deterioration of renal function after 4 years of mild renal failure. Acute tubular necrosis was found in this patient (see Fig. 9-13). The changes in other components of the kidney in this patient were not different from those in other patients with severe hypertension who initially presented with uremia (for details, see patient #5 in Chapter 9).

OTHER CAUSES OF ACUTE RENAL FAILURE

Lithium Poisoning

Because of the increasing use of this agent as a popular antipsychotic drug, it is worthwhile to include this condition separately. Olsen (1976) has studied renal histopathology by LM in five patients with lithium poisoning. No discernible change was found in four of these patients, and focal tubular damage and interstitial infiltrates were found in the remaining one. Electron microscopy was not done; therefore, the validity of this observation should be accepted with reservation.

Nephrotic Syndrome

Oliguria and azotemia are not uncommon in nephrotic syndrome. In childhood, nephrotic syndrome secondary to lipoid nephrosis and associated with oliguria and azotemia is most likely to be caused by decreased intravascular volume. This results from low oncotic pressure owing to hypoalbuminemia and migration of intravascular fluid into the interstitial space. The total effect is the fall of GFR. Treatment with corticosteroids decreases proteinuria, increases serum albumin concentration, and restores serum oncotic pressure to normal. The result is an abrupt increase of GFR and a consequent high urine output and decline of azotemia.

ACUTE RENAL FAILURE CAUSED BY BILATERAL RENAL VEIN THROMBOSIS

Acute renal failure owing to bilateral renal vein thrombosis may occur in membranous glomerulonephritis, amyloidosis, or diabetic glomerulosclerosis. Acute bilateral renal vein thrombosis is heralded by a sudden onset of oliguria, abdominal discomfort or pain, gross or microscopic hematuria, and azotemia. A suspicion of acute bilateral renal vein thrombosis becomes a probability if massive enlargement of the kidneys is demonstrated in a flat film of the abdomen. The diagnosis can be confirmed by bilateral renal venography. Treatment with heparin may be tried, since such treatment has been reported to reverse acute renal failure.

RENAL PAPILLARY NECROSIS

Acute renal failure caused by papillary necrosis has been reported in analgesic nephropathy, diabetic nephropathy, and chronic pyelonephritis, although the incidence is highest in diabetic nephropathy. One obvious reason for this high incidence is the associated acute and chronic pyelonephritis in diabetic patients. For other causes of papillary necrosis in diabetic nephropathy, see the discussion on diabetes mellitus in Chapter 7. It has been proposed that acute renal failure in papillary necrosis is akin to acute obstructive renal failure. Apparently, the sloughed-off papillae block the ureters and produce oliguria or anuria, although whether or not this statement is true is difficult to determine. For instance, how do papillae slough off simultaneously from both kidneys to produce bilateral ureteral obstruction and oliguria? Therefore, it is more likely that mechanisms other than ureteral obstruction alone are the cause of oliguria. In any event, a patient may present with colicky abdominal pain, fever, and shaking chills in addition to oliguria or anuria. Laboratory studies often reveal leukocytosis and pyuria, which indicate that active urinary tract infection may have contributed to this acute condition (papillary necrosis).

A cystoscopy and ureteral catheterization will be diagnostic should papillae be recovered from the ureters. Short of urologic intervention, all urine should be collected and strained through gauze to recover the papillae. All tissuelike material should be fixed, embedded, and studied by light and/or electron microscopy.

HOW RENAL BIOPSY STUDY MODIFIES THE COURSE AND MANAGEMENT IN ACUTE RENAL FAILURE

The diagnosis of acute tubular necrosis is not as clinically obvious as the glomerular diseases. Although it poses fewer problems to recognize poststreptococcal glomerulonephritis or lupus proliferative glomerulonephritis because of the striking clinical manifestations and positive serological tests, it is frequently

difficult to make a diagnosis of rapidly progressive glomerulonephritis solely on the basis of clinical presentations. Therefore, there is a more imperative need for renal biopsy in acute renal failure caused by a suspected glomerular disease than in cases of acute renal failure without it. The histopathological study is essential, especially because the treatment and course differ in different types of glomerular diseases. The value of renal biopsy in suspected tubular lesions is not so important for the diagnosis as for the evaluation of the course. Since the diagnosis and assessment of the degree of tubular lesions are difficult, if not impossible, by clinical and laboratory tests, renal biopsy becomes an absolute necessity. The biopsy is imperative if the patient fails to show signs of recovery after an optimum period (3 weeks) from the onset of the disease.

Some data from published reports are presented to substantiate the values of renal biopsy studies in acute renal failure.

1. Lewers and associates (1970) studied serial renal function (creatinine clearance: C_{cr}, ml/min) and histology in 30 patients who survived acute tubular necrosis for a period of 2 to 15 years. The average duration of follow-up was 9.3 ± 3.6 years. The causes of acute tubular necrosis are shown in Table 8-5. The mean duration of oliguria in these patients was 11.1 ± 6 to 7 days with a mean of 1.4 dialyses per patient; clinical recovery was complete in all patients. Follow-up histopathological studies from some of these patients were described in the section on the evolution of histological repair in ATN. The clearance studies in

Table 8-5
Causes of Acute Tubular Necrosis in the Study Group

	Number of patients
Ischemic	
Transfusion reaction	6
Obstetrical	3
Abruptio placenta	
Eclampsia	
Placenta previa	
Septicemia	1
Shock	5
Postoperative	1
Crush injury	1
Unknown (probably dehydration)	1
Total	18
Toxic	
Carbon tetrachloride	5
Mercury	4
Ethylene glycol	2
Fertilizer compound	1
Total	12

Source: From Lewers *et al.* (1970), by the kind permission of the authors.

ischemic and toxic types of tubular necroses were related to the duration of follow-up (Fig. 8-21). Ischemic acute tubular necrosis was followd by an abnormal creatinine clearance in 44% of cases, compared to 25% in those with toxic cause. However, the difference was not significant (Fig. 8-21). The authors indicated that those who recover become clinically normal and demonstrate reversal of renal histology to normal. In this study, the majority of patients probably reached their maximum clearances during the first year after acute tubular necrosis and then remained relatively constant. The authors observed deterioration of renal function in occasional cases.

2. Wilson and colleagues (1976) performed renal biopsies in 84 patients with acute renal failure. The objective of the renal biopsies was to determine the anatomical diagnosis, especially when renal diseases other than acute tubular necrosis were suspected. Of 67 biopsies, only 14 had renal morphology consistent with ATN. In most of the remaining patients, renal histopathology showed glomerular diseases. The recommendations for renal biopsy in acute renal failure by the authors fully agree with ours and include prolonged oliguria when ATN has been suspected clinically, when a renal lesion other than ATN is under consideration from the clinical standpoint, and assessment of prognosis and plan for management. Another indication is the confirmation of clinical diagnosis. The

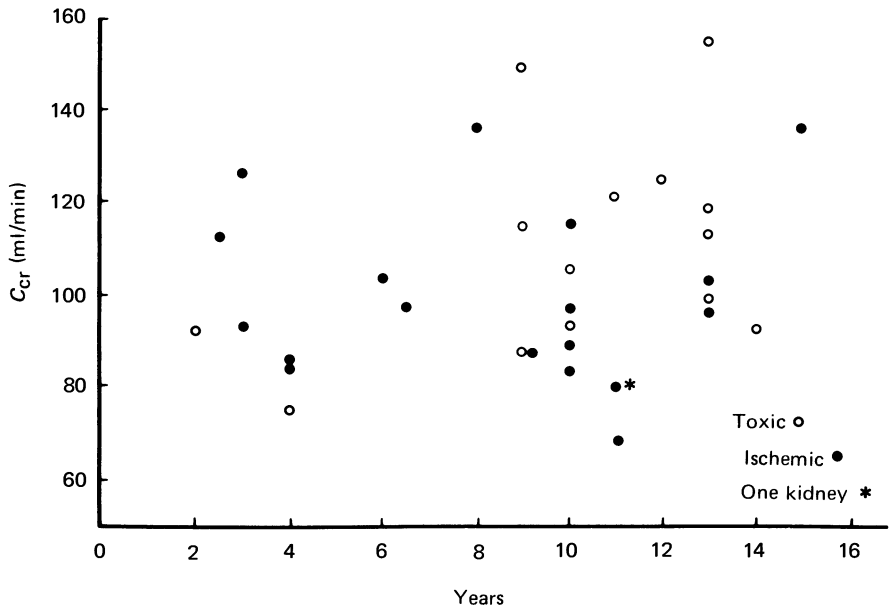

Fig. 8-21. Long-term correlation between renal function (C_{cr}) and histopathologic types (toxic or ischemic) of acute tubular necrosis after recovery. Toxic type appeared to have greater functional recovery (average C_{cr} 110.3 ml/min) than ischemic type (average C_{cr} 99.5 ml/min). The difference was not significant. From Lewers *et al.* (1970), by the kind permission of Dr. George E. Schreiner.

Table 8-6
How Renal Biopsy Study Modifies the Course and Management in Acute Renal Failure

Morphological diagnosis	Course	Long-term management plan
Acute renal tubular lesions (mild and without thrombosis)	Recovery (generally)	Generally none, follow-up
Complicated acute renal tubular lesions (acute tubular necrosis with thrombosis)	Recovery (less common)	Generally none, follow-up, hemodialysis in small percentage of cases
Acute glomerulonephritis		
Immune complex type	Recovery or progression to chronic renal failure	Corticosteroids, immunosuppressives, hemodialysis program, transplantation
Anti-GBM antibody type (Rapidly progressive glomerulonephritis)	Irreversible	Hemodialysis, transplantation
Acute interstitial nephritis	Recovery (usual)	None or corticosteroids
Acute cortical necrosis	Irreversible	Hemodialysis, transplantation
Renal lesions of hypertension (malignant renal lesions)	Partially reversible or irreversible	Antihypertensive drugs, hemodialysis, transplantation

most distinctive values of renal biopsy study in the clinical care of acute renal failure are summarized in Table 8-6.

SUMMARY

1. Acute renal failure (acute uremia) is a syndrome resulting from lesions of one or more components of the kidney.

2. Prompt recognition of the syndrome and rapid implementation of adequate management to reduce the mortality is more important than attempting to delineate the cause at the beginning.

3. Renal biopsy may be deferred if sound clinical background (e.g., trauma, shock, hemolysis) suggests ATN.

4. Renal biopsy should not be withheld if there is strong suspicion of glomerular disease. More importantly, renal biopsy becomes imperative should the patients fail to show signs of recovery (i.e., increase of urine output, decrease in serum urea nitrogen and serum creatinine) 3 weeks after the onset of the disease.

5. In general, the vast majority of cases of acute renal failure are caused by acute tubular lesions. In most cases, the morphological changes are mild and should be described appropriately as renal tubular lesions. The term acute tubular necrosis should be reserved to denote definite necrotic changes. Therefore, def-

inition of the tubular changes is mandatory and can be achieved accurately only by EM study using the silver technique (PAMS).

6. Although there is a difference in the severity of morphological changes between acute tubular lesions and ATN, no significant difference is observed in survival and clinical course.

7. Although it is easy to recognize renal tubular lesions, the pathogenetic mechanism for the tubular lesions and the relationship between the tubular lesions and the oliguric syndrome remain obscure. A variety of experimental models have been studied in order to explain the mechanism; none is 100% satisfactory, but initiation by ischemia (decreased renal blood flow) and perpetuation by intrarenal vascular thrombosis appear to be plausible concepts. Oliguria is most likely to be caused by decreased glomerular filtration, tubular obstruction, and tubular backdiffusion of the glomerular filtrate.

8. The theory concerning acute interstitial nephritis as a cause of acute renal failure is becoming popular. It is not yet established whether the clinical syndrome can be attributed to the interstitial cellular infiltrates (acute interstitial nephritis) alone, to accompanying tubular lesions alone, or to both. It is reasonable to state that this entity is acute tubulointerstitial nephritis rather than acute interstitial nephritis.

9. Prompt recognition and management of the initiating or precipitating factors can avert further renal damage—for example, correction of intravascular volume (ATN), withdrawal of the offending therapeutic agents (AIN), control of urinary tract infection (papillary necrosis), and correction of proteinuria (nephrotic syndrome).

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9 Pathology of the Kidney in Essential (Spontaneous) Hypertension

INTRODUCTION

The anatomic pathology of the kidney in hypertension and the relationships between the clinical entity and the morphological changes in cortex and medulla of the kidney are presented in two separate parts. In Part 1, the changes in different components of the cortex and their relationship to different types of clinical hypertensions are described. The data from experimental studies are presented to clarify ambiguities. In Part 2, analyses of papillary interstitial cells and granules in normotensive humans and animals and in hypertensive animals are described. The role of the papillary interstitial cells and granules in guarding against hypertension is explored.

PART 1
CAUSE AND EFFECT RELATIONSHIP BETWEEN RENAL LESIONS AND HYPERTENSIONS

HYPERTENSIVE RENAL LESIONS

SYNONYMS. Benign nephrosclerosis, malignant nephrosclerosis

The renal pathology associated with essential hypertension has been labeled arteriolar nephrosclerosis. Nephrosclerosis is divided into benign and malignant types. It is considered benign when the small arteries and arterioles exhibit hyaline (eosinophilic and PAS-positive) thickening of the vessel wall with slight

or no change in other components of the kidney, and malignant when it is characterized by necrotic and/or proliferative changes in the large and small arterioles, necrosis and hyalinization in the glomeruli, a decrease in the number of tubules, and variable infiltration of the interstitium by lymphocytes and fibrous tissue. Although the term *vascular lesion* is used customarily to denote renal lesions in hypertension, *renal lesion* is used throughout the chapter to indicate lesion in the arterial vessels and other components of the renal cortex in hypertension.

Many investigators have reported on the fine structure of the renal arterioles in benign essential and malignant hypertension; however, these studies have failed to delineate the morphogenesis of the renal lesions and the importance of the blood pressure elevation in the development of the observed changes. Although much of this chapter deals with the morphological changes observed in the kidneys of patients with benign essential and malignant hypertension, and in spontaneously hypertensive rats, three additional objectives are pursued: (1) to demonstrate a relationship between the extent of renal lesions and both severity and duration of the hypertension; (2) to delineate the relationship of the morphological changes to renal failure, i.e., is the renal failure caused by the lesion of the arterial vessels alone, as is generally believed, or is it the result of a more diffuse lesion in the kidney; and (3) to highlight the value of electron microscopic studies for our understanding of the pathology and morphogenesis of the renal lesions associated with hypertension.

Human Study

The kidneys from 10 hypertensive patients were studied. The pertinent clinical and laboratory information is shown in Tables 9-1 and 9-2, respectively. Renal tissues were obtained by either percutaneous or open renal biopsies and after nephrectomies. In two patients (#5 and #7), two separate studies were performed, at intervals of 43 months and 12 months, respectively, between biopsies and nephrectomies. Complete laboratory and radiological evaluations were done to exclude secondary forms of hypertension in all patients.

Rat Study

Kidneys from 46 spontaneously hypertensive rats (SHR), 35 normotensive Wistar rats (NR), and 28 normotensive Wistar Kyoto rats (WKY) of similar age distribution (8–104 weeks) were studied using light microscopy (LM) and electron microscopy (EM). Rats were divided into young (<52 weeks) and old (≥ 52 weeks). An experiment was carried out in which 10 (7 old, 3 young) SHR were treated with aqueous heparin 150 units (0.15 ml) twice daily for 30 days. An equal number of rats received an equivalent amount of normal saline for the same duration. The kidneys from these rats were studied by immunofluorescence microscopy (IFM) in addition to the studies by LM and EM (for details of LM

Table 9-1
Clinical Features in Hypertensive Patients

Patient number	Age and sex	Presenting symptoms	Blood pressure (mm Hg)	Duration of hypertension (years)	Funduscopic findings (K-W-B grading)
1	14M	Hematuria	170/100	2	0
2	39M	Weakness	210/110	6	II
3	22M	Vomiting	220/130	2	II
4	51M	Recurrent chest pain	140/110	23	II
5	25M	Headache	240/140	Unknown	II
6	33F	Headache and ecchymosis	160/110	6	I
7	23M	Headache and faintness	240/140	Unknown	IV
8	33M	Headache	230/130	Unknown	IV
9	32M	Urinary tract infection	230/130	Unknown	IV
10	19M	Swelling of tongue	240/140	2	IV

Source: From Mandal *et al.* (1977*c*), by the kind permission of the editors.

and EM techniques, see Chapter 2). The technique for immunofluorescence microscopy study is described here. In all rats, direct mean arterial blood pressures (MAP) were measured under light ether or pentobarbital anesthesia, administered via femoral artery catheterization, and then the animals' kidneys were removed.

Immunofluorescence microscopy study. For IFM, tissues were frozen at −20°C in an embedding medium. Then, 4-μm sections were cut with the cryostat, placed on slides, washed twice for 5 min each by immersion in phosphate-buffered saline (PBS) at pH 7.2, and finally fixed in acetone for 12 min. Fluorescence isothiocyanate-conjugated rabbit antisera against rat IgG, gamma globulin (γG), fibrinogen, third component of complement (C3), and albumin were added to five separate slides prepared from each kidney. The slides were incubated for two consecutive periods of 45 min each, at first in a humidified chamber at 37°C, then at room temperature, and afterwards were washed thoroughly with PBS to remove excess stain. They were mounted with buffered glycerine (90 parts glycerine to 10 parts PBS) and studied using a Leitz-Dialux microscope with ultraviolet light. The tissues stained with antialbumin serum provided the control for the immunoglobulins and fibrinogen.

Detailed Clinical Information and Anatomic Pathology of the Kidney

By light microscopy, kidneys from the first four patients (Table 9-1) demonstrated a normal appearance in most of the glomeruli, tubules, and interstitium; the large and small arterioles showed a normal appearance or thickening of the intima by PAS-positive matrix and thinning of the media. All arterioles were essentially hypocellular (Figs. 9-1 and 9-2). There was no evidence of necrotic change in any component. By electron microscopy (EM), the arterioles revealed

Table 9-2
Laboratory Information in Hypertensive Patients

Urinary protein[a]	Serum urea nitrogen (mg/100 ml)	Serum creatinine (mg/100 ml)
0	12	0.9
4+ (0.5)	48	2.6
4+ (0.35)	22	1.6
4+ (0.12)	26	1.6
4+	27	2.3
4+	84	10
4+	110	13
4+	100	12
4+	139	21
4+ (0.6)	112	13

[a] Numbers in parentheses are the 24-hr urinary protein.
Source: From Mandal *et al.* (1977*c*), by the kind permission of the editors.

thickening of the basement membranes (BM) between endothelial cells and smooth muscle cells (SMC) and between individual smooth muscle cells, excessive BM-like material throughout the arteriole, and normal or atrophic SMC (Figs. 9-3 to 9-5).

Patient Illustration

Patient #5, E.P., a 25-year-old-black male, had numerous admissions to the Oklahoma City VA Hospital for investigation and management of hypertension. He presented to the general medicine clinic first in November 1972 with the complaint of a persistent headache for 4 days and was found to have blood pressure 240/140 mm Hg and grade II (K-W-B) retinopathy. Serum urea nitrogen and serum creatinine concentrations were 27 and 2.3 mg/100 ml, respectively. A complete workup including exploratory laparotomy

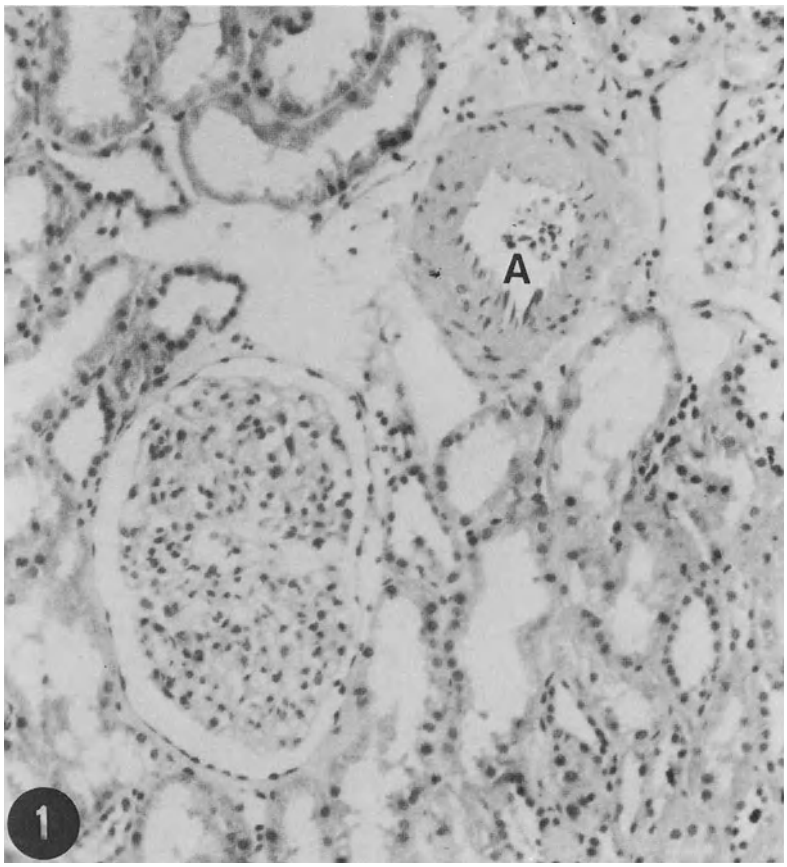

Fig. 9-1. Normal appearance of the glomerulus and the tubules. The large arteriole (A) shown appears to be within normal limits (H & E, ×120). From the renal biopsy of patient #1 in Table 9-1.

failed to demonstrate an underlying cause for hypertension. A wedge biopsy of the kidney was taken during exploratory laparotomy and renal tissue was studied using LM, EM, and IFM. The patient was placed on antihypertensive drugs; his headache promptly disappeared, and the serum urea nitrogen and serum creatinine fell to near normal values. On routine visits to the Renal Clinic, he was found to have marked elevations of blood pressure, in excess of 240/140 mm Hg on numerous occasions, many of which warranted short hospital admissions for control of the blood pressure. However, the serum urea nitrogen and serum creatinine concentrations were always below those observed at his first admission. After 36 months, he had a sudden onset of exertional dyspnea, followed by paroxysmal nocturnal dyspnea and oliguria, and was admitted to the hospital. His blood pressure was in the range of 250–260/140–150 mm Hg and the fundi showed grade

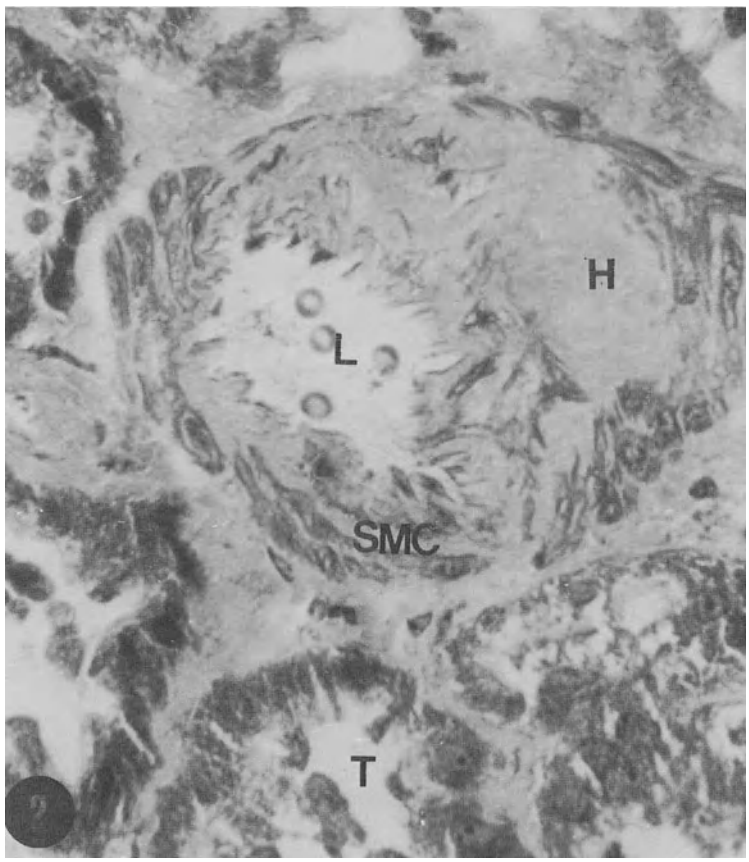

Fig. 9-2. In this large arteriole, one-half of the media (smooth muscle cell layer) has been replaced by a mass of homogeneous material (H) which has been variously described as hyaline material, hyalinosis, and so on. Smooth muscle cells (SMC) and the lumen (L) of the arteriole are shown (H & E, ×320). From the same specimen as Fig. 9-1.

III (K-W-B) retinopathy. He had rapid deterioration of renal function with an increase in serum urea nitrogen and serum creatinine concentrations to 110 and 11.5 mg/100 ml, respectively. After treatment with large doses of antihypertensive drugs, including intravenous administration, along with digitalis and diuretics, his blood pressure was controlled and the serum urea nitrogen and creatinine concentrations decreased to 80 and 8.5 mg/100 ml, respectively. Eight weeks following this episode, his renal function again deteriorated, blood pressure increased, and he was placed on the maintenance hemodialysis program.

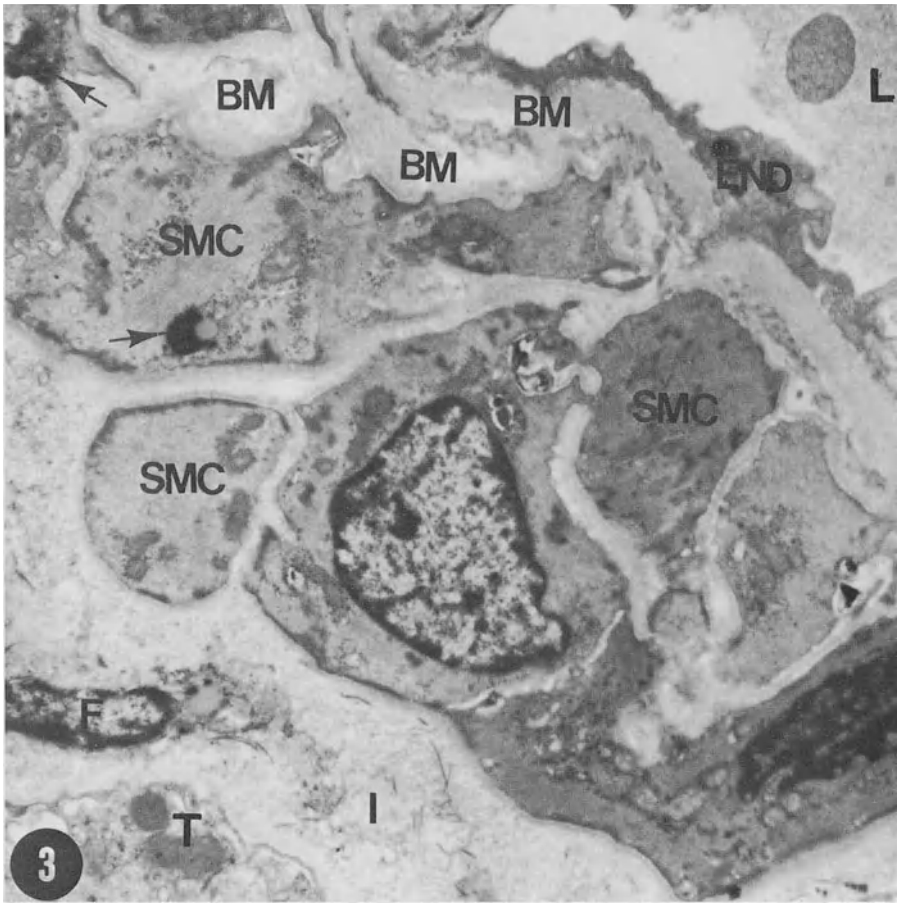

Fig. 9-3. In this large arteriole, mild to moderate thickening of the basement membrane (BM) between endothelial cell (END) and smooth muscle cells (SMC), excessive BM-like materials, and atrophy of most SMC and END are seen. Atrophic cells are characterized by small nuclei, a few cell organelles, and the appearance of lysosomes (arrows). Lumen of the arterioles (L) is shown. The outer border merges into the interstitium (I), a tubule (T), and a fibroblast (F) (UA + LC, ×12,000). From the same specimen as Fig. 9-1.

Despite treatment with hemodialysis and massive doses of antihypertensive drugs, his blood pressure continued to rise. He underwent bilateral nephrectomy 43 months after the initial biopsy and the renal tissue was studied by LM, EM, and IFM.

Biopsy Specimen

The renal biopsy specimen showed essentially normal glomeruli, a loss of 5 to 10% tubules, a few atrophic tubules, mild interstitial fibrosis, and moderate to marked thickening of the intima or hyperplasia of SMC in the large arterioles as well as thickening of the intima in the small arterioles (Fig. 9-6). The so-called thick sections (0.5 μm), stained with methylene blue and azure II and studied by LM, revealed marked SMC hyperplasia (Fig. 9-7) Glomeruli showed moderate to

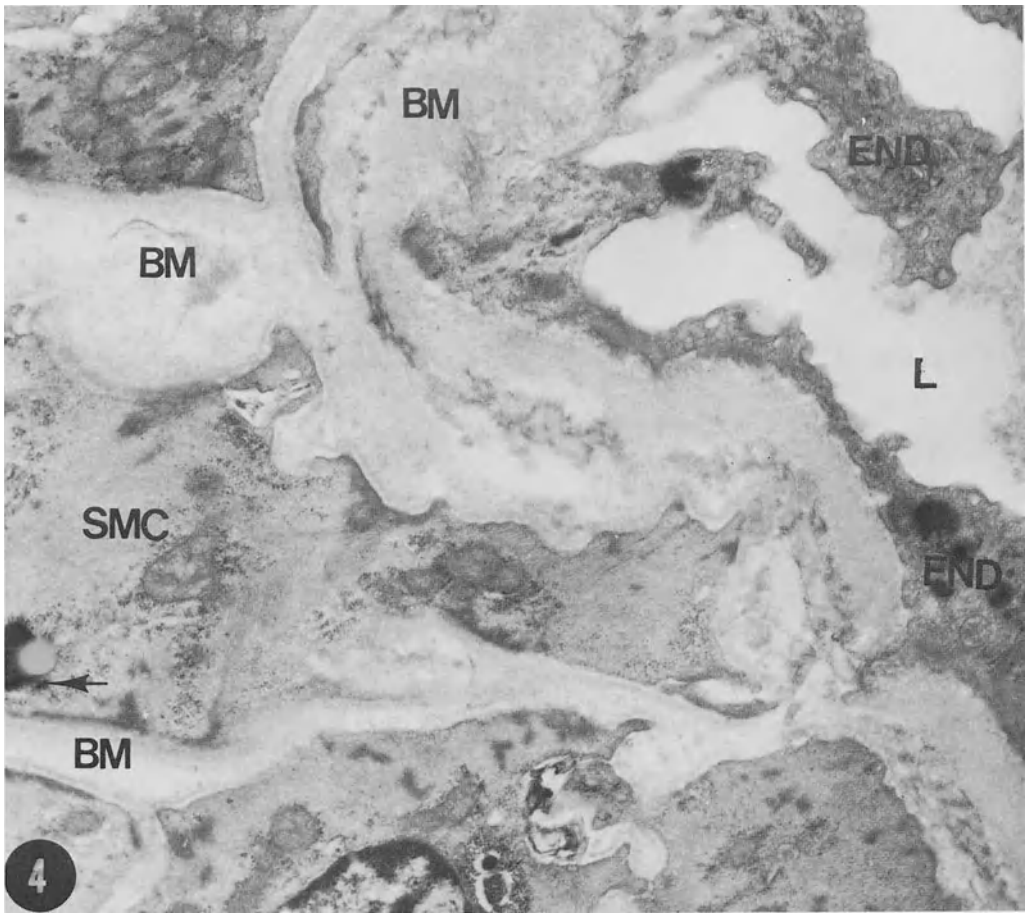

Fig. 9-4. Magnified view of a large arteriole in which thickened basement membranes (BM) and atrophic smooth muscle cells (SMC) are shown. Lysosomes within SMC (arrow), lumen of the arteriole (L), and endothelial cell (END) are shown (UA + LC, ×18,000).

marked irregular thickening of the peripheral capillary loops (Fig. 9-8). By EM, the arterioles revealed intact endothelial cells, hyperplasia, atrophy and/or necrosis of SMC, a few fibroblasts, small amounts of collagen fibers, and electron-dense deposits (Fig. 9-9). There were increased amounts of electron-dense elastic tissue. Glomeruli showed irregular thickening and tortuosity of basement membranes (GBM), small discrete deposits of electron-dense materials within GBM, and crescent formation.

Nephrectomy Specimen

The LM study (Figs. 9-10 to 9-13) revealed more severe changes than those in the biopsy specimen. Some arterioles demonstrated marked intimal thickening,

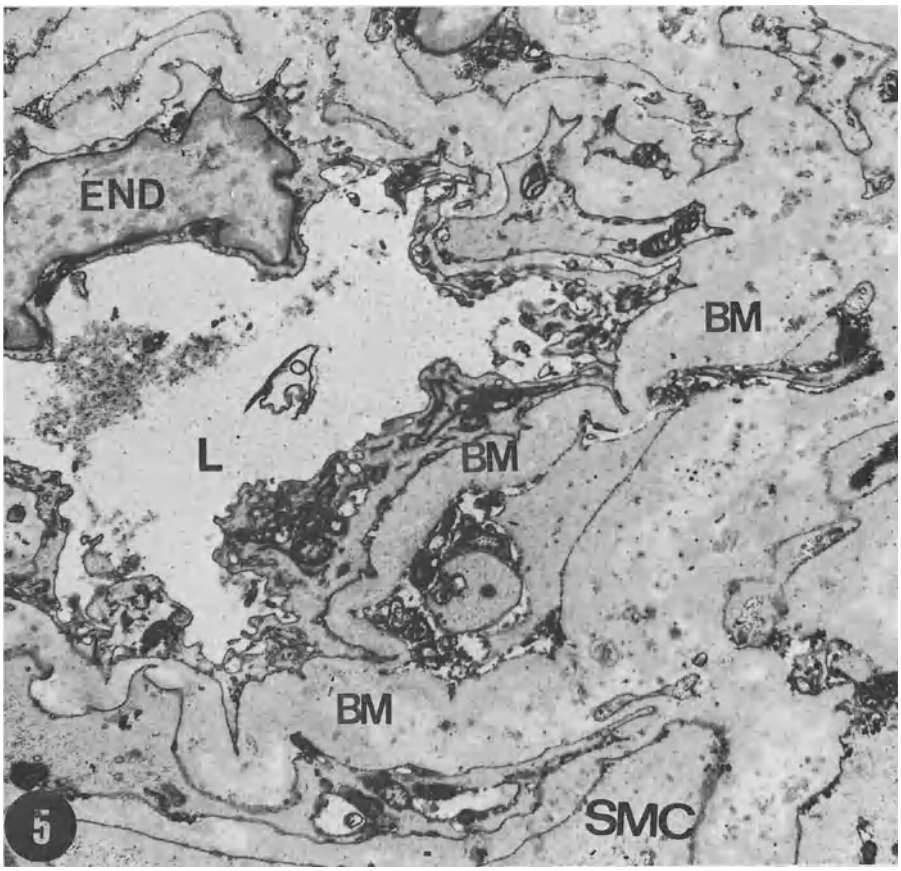

Fig. 9-5. After the application of a special stain, this large arteriole shows atrophy of endothelial cells (END), moderate to marked thickening of the basement membrane (BM) between END and smooth muscle cell (SMC), and marked atrophy of SMC. Lumen of the arteriole (L) is shown (PAMS, ×10,000). From the renal biopsy of patient #2 in Table 9-1.

some marked SMC hyperplasia, and still others loss of endothelial cells and occlusion of the lumina. Intense PAS-positive material suggestive of necrosis in the intima and occlusion of the lumina by thrombi was observed in some arterioles. Rarely, aneurysmal bulging through a disrupted segment of large arteriole produced a conspicuous histological feature (Fig. 9-12). The periphery of this arteriole revealed excessive periadventitial fibrous tissue without inflammatory infiltrates. About 60% of the glomeruli revealed marked, irregular thickening of the peripheral capillary loops. Twenty percent of the glomeruli were hyalinized and revealed fibrotic crescents; an equal number showed necrosis of capillaries and plasma material into Bowman's space. Few glomeruli appeared normal. The

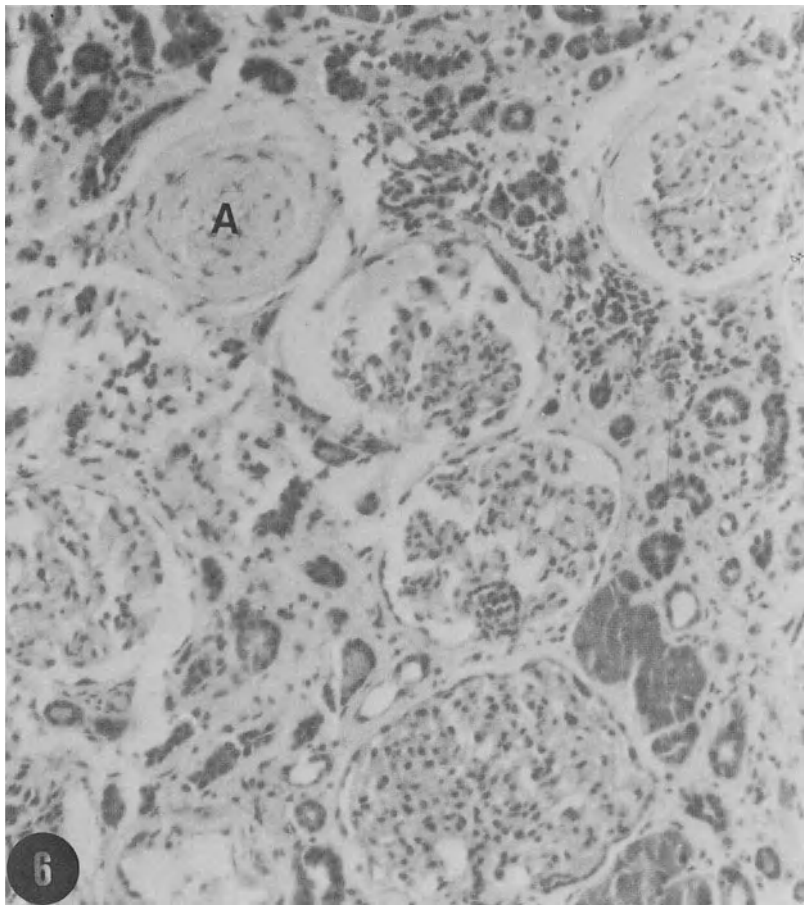

Fig. 9-6. This micrograph shows slightly hypercelluiar glomeruli, especially the large one at the bottom, fibrosis of the Bowman's membrane at the upper right glomerulus, and scarcity of tubules. The large arteriole (A) reveals a cellular thickening with complete occlusion of the lumen (H & E, ×120) From the renal biopsy of patient #5 in Table 9-1.

tubules were variable, some being normal, some atrophic, and others dilated and filled with casts. Ten to twenty percent of the tubules showed necrotic changes and a few tubules were filled with red cells (Fig. 9-13).

Electron microscopy studies of arterioles demonstrated predominant hyperplasia of SMC in one arteriole to mainly necrosis of SMC in another arteriole (Figs. 9-14 to 9-16). The hyperplastic SMC were characterized by a large, notched, or bifid nucleus or multiple nuclei, numerous ribosomes, and many

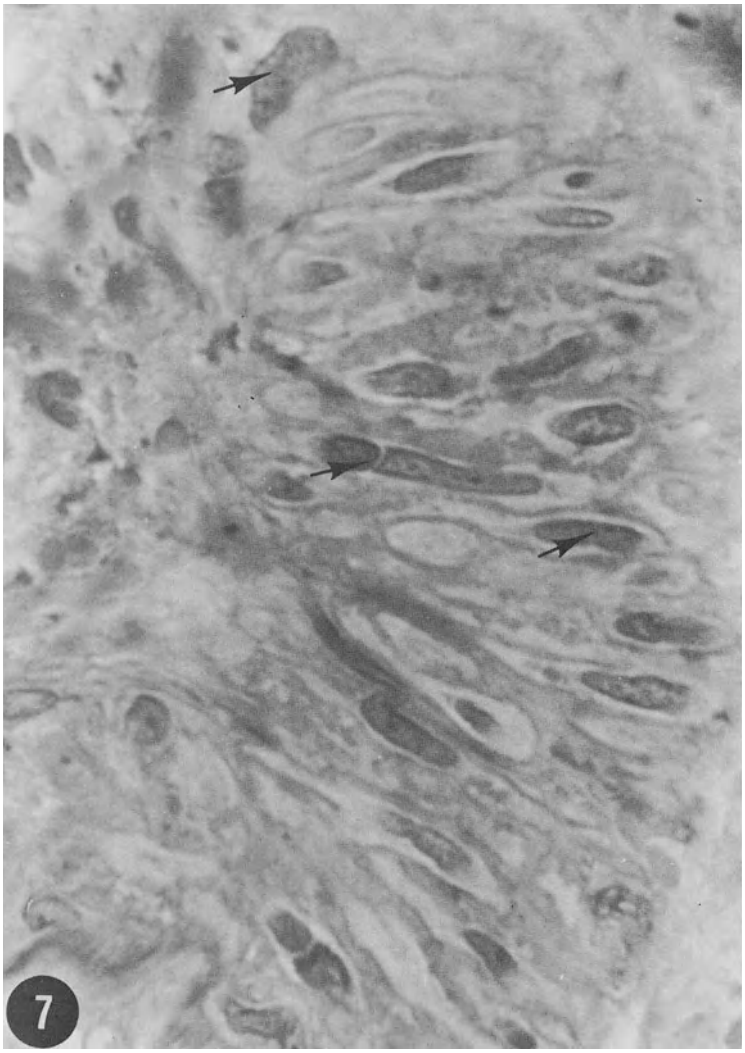

Fig. 9-7. Proliferation of smooth muscle cell evidenced by large or double nuclei (arrows). Survey section (so-called thick section) of an arteriole is shown (methylene blue–azure II, ×800). From the same specimen as Fig. 9-6.

dilated endoplasmic reticula. Along with hyperplastic SMC, a few atrophic SMC were also observed. In general, the arteriolar changes were more severe and widespread than those observed in the biopsy specimen. EM of glomeruli revealed irregular but pronounced thickening and tortuosity of GBM in most cap-

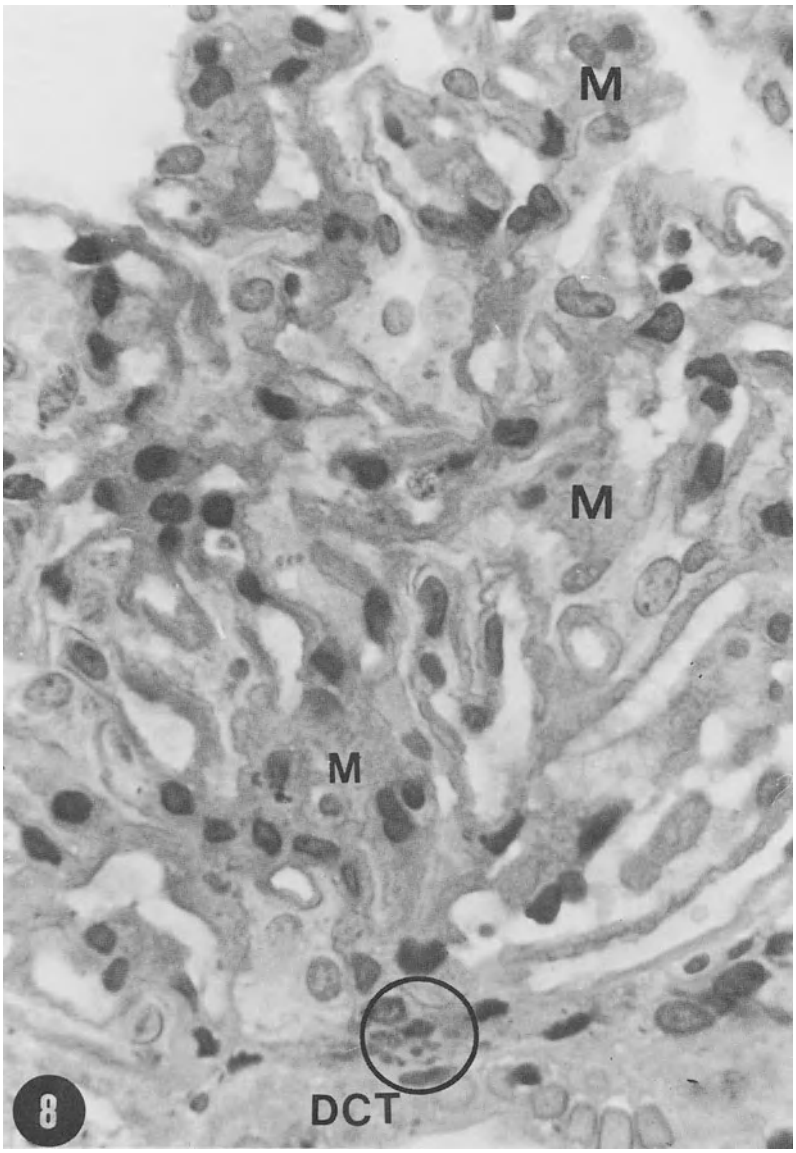

Fig. 9-8. Diffuse and marked thickening of the basement membrane of the peripheral capillary loops and moderate increase in mesangial matrix (M). The juxtaglomerular apparatus is circled. The dark spherical structures within the JGA may be granules or mitochondria. Distal tubule (DCT) is shown (survey section stained with methylene blue-azure II, ×800). From the same specimen as Fig. 9-6.

illaries, necrosis of some capillaries, discrete electron-dense deposits, atrophy of endothelial cells, crescent formation, or exudation in Bowman's space. IFM studies were negative for immunoglobulins in both biopsy and nephrectomy specimens. However, fine granular deposits of C3 were found in the walls of small arteries along with dense deposits of fibrinogen in occasional small arteries.

In summary, two separate histological studies of renal tissue by LM, EM, and IFM at an interval of 43 months revealed striking progression of morphological changes in the nephrectomy specimen. The changes were characterized by a marked increase in hyperplastic SMC and increases in collagen fibers and elastic tissue in most arterioles, by necrosis of SMC in a few arterioles, by

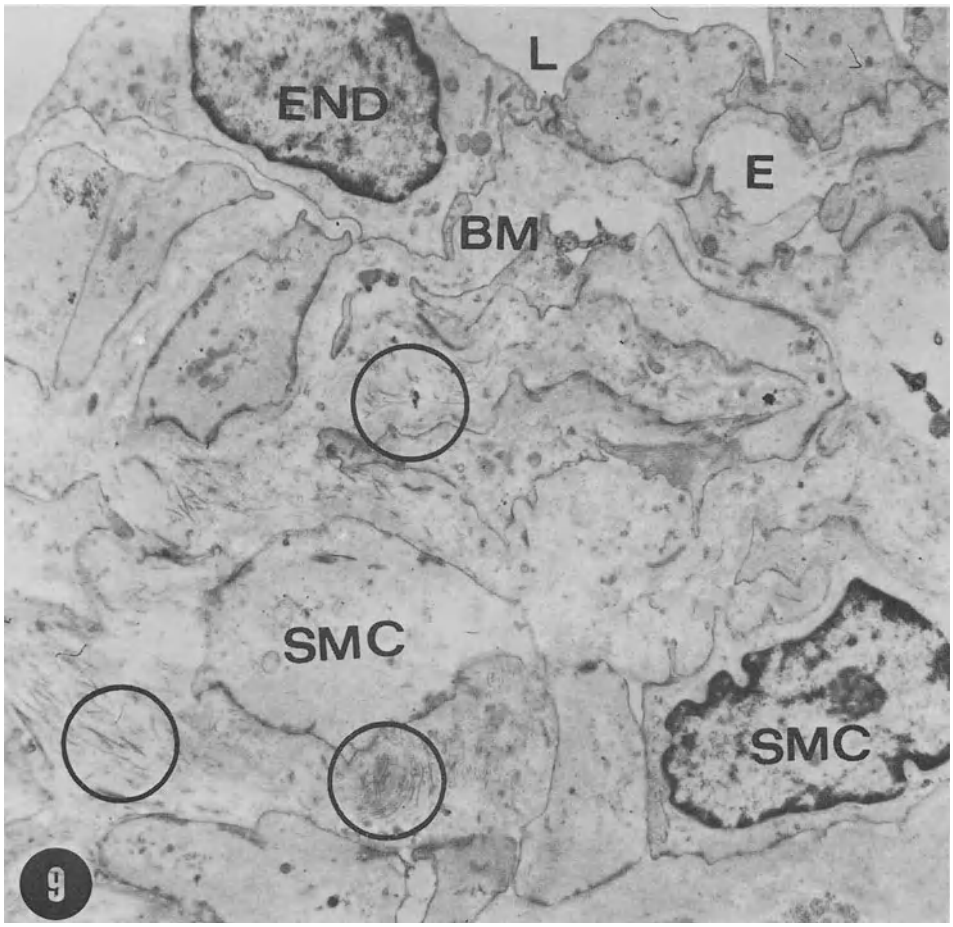

Fig. 9-9. Necrosis and edema (E) of the basement membrane (BM) between endothelial cell (END) and smooth muscle cell (SMC), necrosis of most SMC, and excessive amount of collagen fibers (circles) throughout the arteriolar wall can be seen. Lumen of the arteriole (L) is shown (UA + LC, ×12,000). From the same specimen as Fig. 9-6.

necrosis and hyalinization in 20 to 25% of glomeruli, by atrophy and dilatation of most tubules, by frank necrosis of 10 to 20% of tubules, and by a conspicuous increase in interstitial infiltrates and fibrosis.

The renal pathology in patients 6 through 10 resembles that in the nephrectomy specimen of patient #5. However, observed differences were an excessive number of fibroblasts and platelets in the intima, predominant hyperplasia of SMC, and an excessive amount of collagen fibers and elastic tissue (Figs. 9-17 to 9-26). These differences were more marked in patients #7 and #10 than in the others. The LM and EM findings of all patients are summarized in Tables 9-3 and 9-4, respectively. The LM pathology separates these patients into two groups: group 1, patients #1 to 4 and biopsy specimen of patient 5; and group 2, neph-

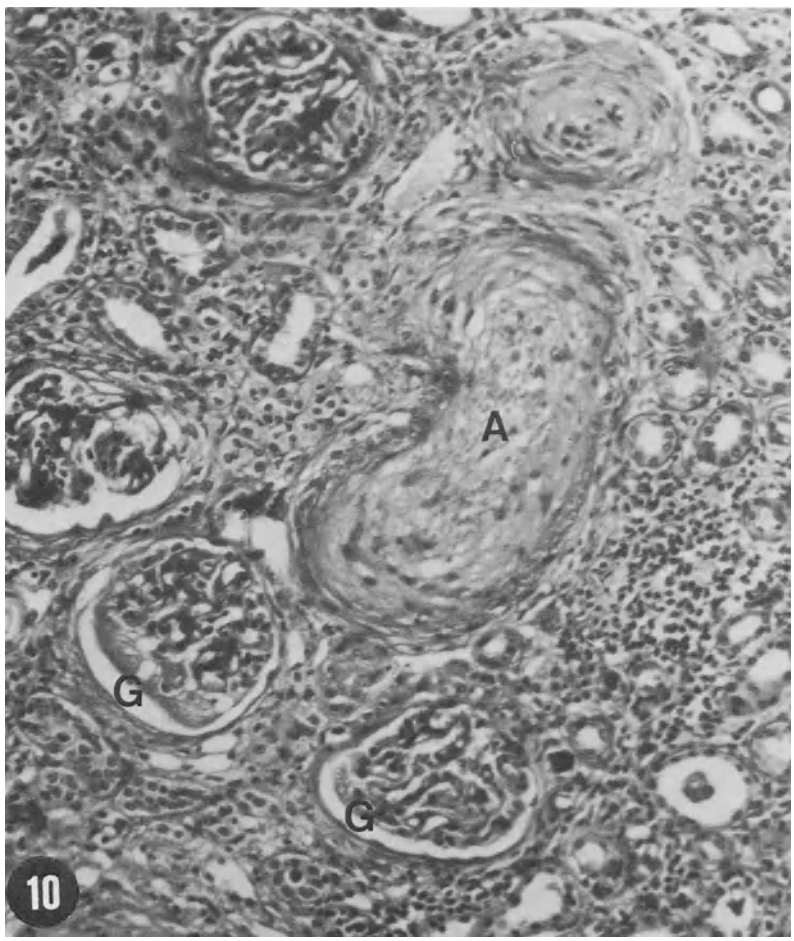

Fig. 9-10. Necrosis of a small artery (A), necrosis of parts of two glomeruli (G), a few atrophic tubules, and marked infiltration of the interstitium by monomorphic round cells are seen (H & E, ×120). From the nephrectomy specimen of patient #5 in Table 9-1.

Table 9-3
Light Microscopy Studies

Group	Patient number	Glomeruli	Arteriole	Tubule	Interstitium
1	1–4	Normal to thickened basement membranes of peripheral capillary loops	Normal or thickening of intima by PAS-positive matrix and thinning of media	Mostly normal, atrophy of 5–10% of tubules	Normal or mild fibrosis of the interstitium
2	5 (nephrectomy)	Hyalinized, necrotic, normal	Hyperplasia and/or necrosis of SMC	Conspicuous scarcity of tubules, atrophy and dilatation of 20–25% of tubules, necrosis of 10–20% of tubules	Moderate to marked fibrosis of the interstitium and foci of infiltration by inflammatory cells
	6–10				

Source: From Mandal *et al.* (1977*c*), by the kind permission of the editors.

rectomy specimen of patient 5 and patients 6 to 10 (Table 9-3). EM study revealed two disparate types of pathology among these patients. The ultrastructural appearances are identical in patients 1 through 4, but unlike in patients 5 through 10 (Table 9-4). EM resolved that changes in the biopsy of patient #5, although mild, otherwise resembled those in the nephrectomy specimen of patient 5 and those in the kidneys of patients 6 through 10.

EXPERIMENTAL STUDY

Studies of the Histology of the Kidney in Rats

The spontaneously hypertensive rat has been considered the best model of its counterpart, the essential hypertensive human. This has been recognized on the basis of some similarities between the origin and course of hypertension in SHR and essential hypertension in humans. These similarities are (1) an heredi-

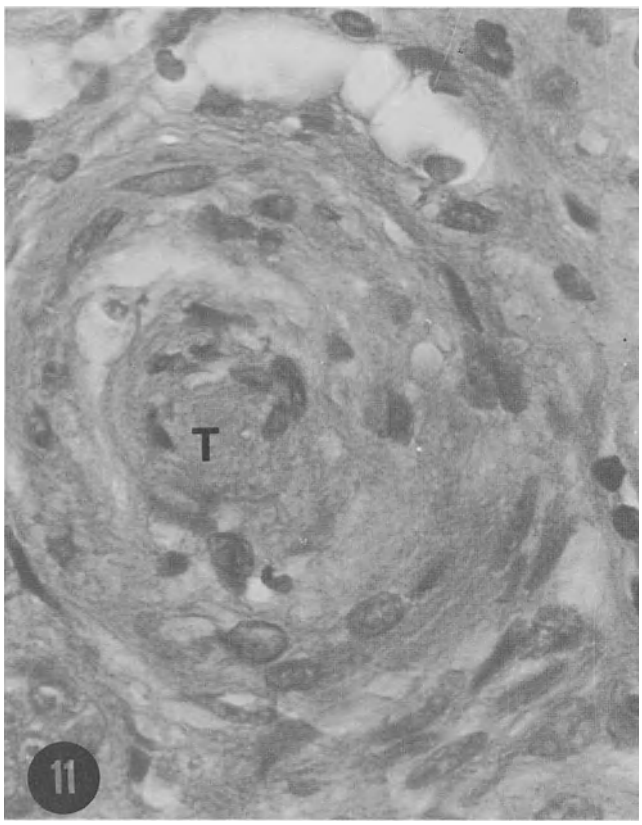

Fig. 9-11. Hyperplasia and necrosis of smooth muscle cells and occlusion of the lumen of a large arteriole by a thrombus (T) are shown (H & E, ×320). From the nephrectomy specimen of patient #5 in Table 9-1.

Table 9-4
Summary of the Ultrastructural (EM) Findings[a]

	Patients 1–4	Patients 5–10
Architecture	N	Distorted
Endothelium	N	Mostly lost
BM of endothelial cell	2+ to 3+ thick	Fibrillar
BM material	2+ to 3+	0
Smooth muscle cell	Normal and atrophic	Necrosis in the inner part, hyperplasia ahd hypertrophy in the outer part
Infiltrations		
Fibrin	0	1+ (infrequent)
Fibroblasts	0	1+ to 2+
Platelets	0	1+
Inflammatory cells	0	1+ to 2+
Collagen fibers	0	3+
Elastic tissue (STPPS)	Occasional	2+ to 3+
Electron-dense deposits	0	Infrequent
Edema	0	2+ to 3+
Glomerular changes	Tortuous, irregular thickening of the basement membrane	Thickening of basement membrane, intramenbranous and subendothelial electron-dense deposits, and necrosis of capillary loops

[a] Scoring: 0 = none, 1+ = mild or few, 2+ = moderate, 3+ = marked, N = normal.
Source: From Mandal *et al.* (1977*b*,*c*), by the kind permission of the editors.

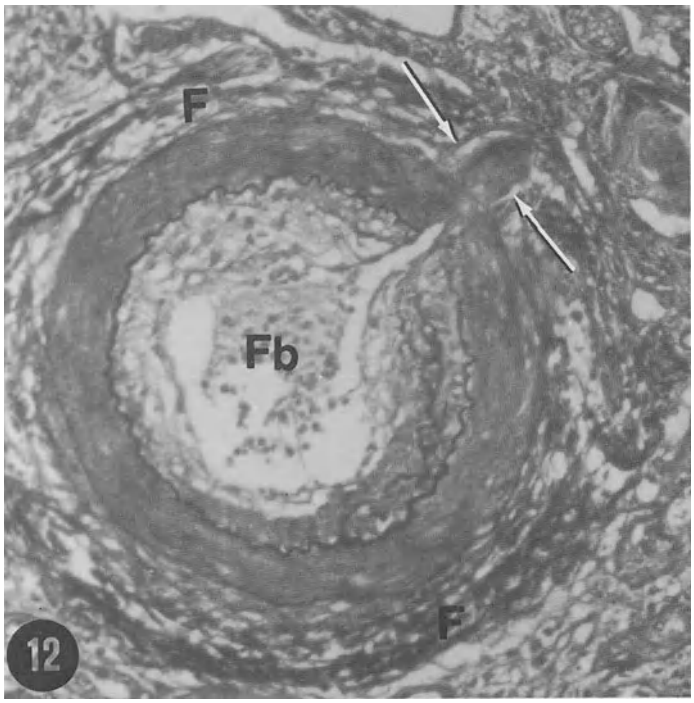

Fig. 9-12. Disruption with aneurysmal dilatation of a segment of this small artery (opposing arrows), with fibroblastic proliferation of the inner aspect (Fb) and excessive fibrosis on the outer aspect (F). A few tubules and fibrosis in the interstitium are seen (Gomori's trichrome, ×320). From the same specimen as Fig. 9-11.

tary component, (2) no evidence of renovascular obstruction, (3) no evidence of neurogenic origin, (4) similarity of the sequelae in SHR to those observed in essential hypertension, and (5) normal or low plasma renin activity.

The renal changes in SHR were compared with those in NR and WKY to avoid overinterpretation and error. The histology of the kidney was evaluated without prior knowledge of strain, blood pressure, or age of the rats. The renal

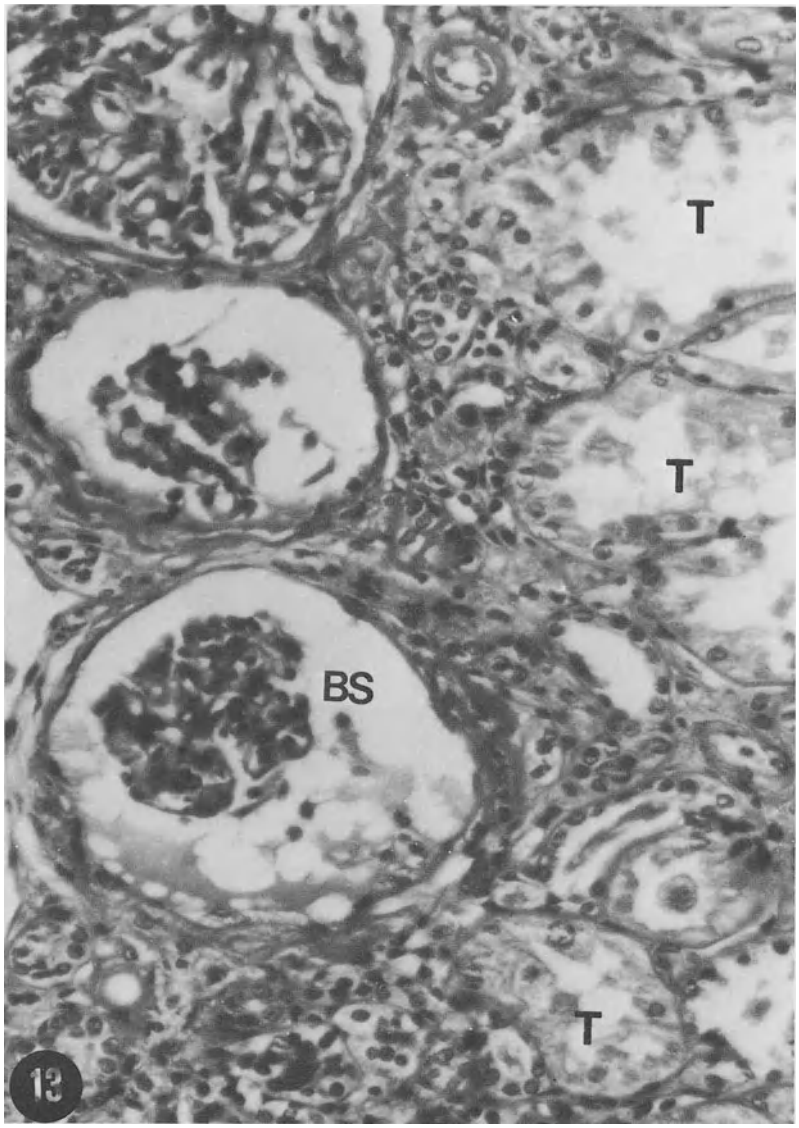

Fig. 9-13. Necrosis of the glomerular tufts and exudation within the Bowman's space (BS) are evident. Diffuse and widespread necrosis of tubules (T) is discerned (H & E, ×120). From the same specimen as Fig. 9-11.

lesions were scored arbitrarily on a scale of 0 to 39, based on the severity of changes observed by light microscopy (index scores). The scoring system is a modification of the method used by Sommers and colleagues (1958) and is listed in Table 9-5. The renal lesions were graded in order of increasing severity: grade I, 0–2 points, normal; grade II, 3–7 points, mild (Fig. 9-27); grade III, 8–19 points, moderate; and grade IV, 20–39 points, severe (see Figs. 9-28 to 9-34 which demonstrate a panorama of malignant renal lesions in old SHR). The grading of renal lesions in terms of severity of hypertension is shown in Table 9-6. The distribution of the rats in each of the three species into these four categories is shown in Table 9-7. The histopathological scores were related to age and blood pressure in the three different strains of rats. It is interesting to note that, except for one WKY, no normotensive rat had grade III or moderate renal lesion, and

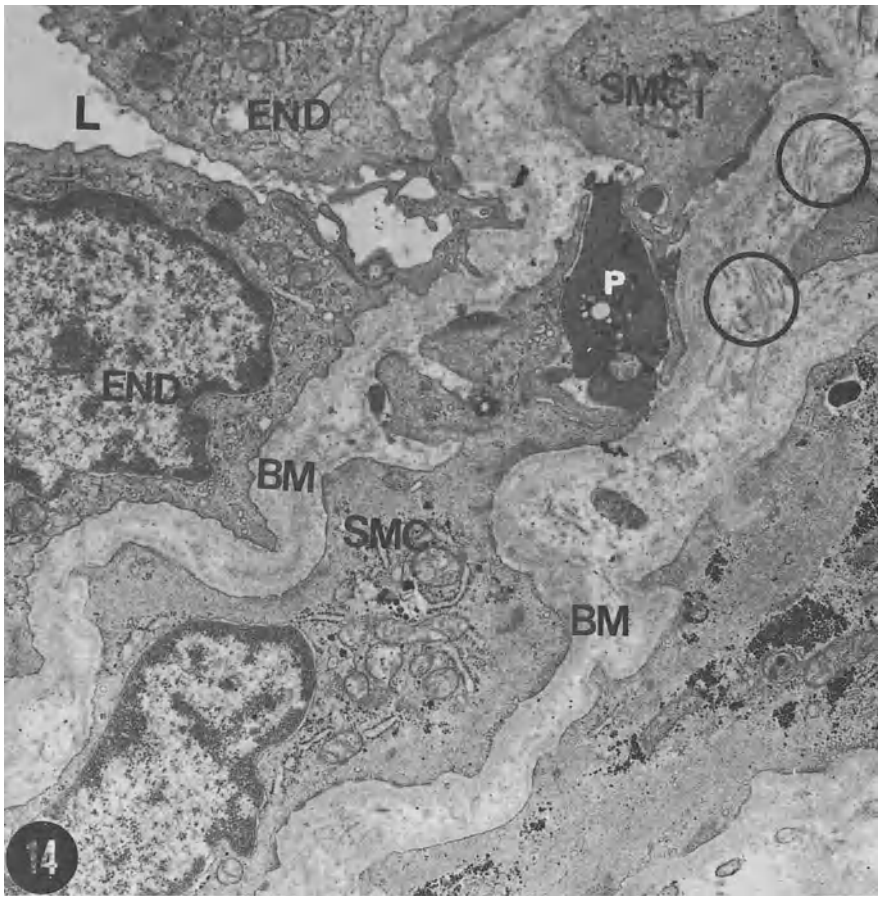

Fig. 9-14. Hyperplasia of a smooth muscle cell (SMC) and atrophic changes in other smooth muscle cells (SMC_1), infiltration by a platelet (P), and excessive collagen fibers (circles) within the basement membrane (BM) between SMC are seen. Lumen (L) of the arteriole and endothelial cells (END) are shown (UA + LC, ×15,000). From the same specimen as Fig. 9-11.

no normotensive rat had grade IV renal lesions (Fig. 9-35). The grade IV or malignant renal lesions were found exclusively in old SHR (average age 91 weeks).

By EM, five different types of changes in the arterial vessels along with thrombi in the glomeruli in SHR have been observed. The appearances in the arterial vessels comprise (1) normal ultrastructure, (2) thickened BM between endothelial cells and SMC and between individual SMC, (3) atrophy of SMC, (4) hyperplasia of SMC, (5) necrosis of BM and SMC. In old SHR only, glomeruli show normal or necrotic GBM, electron-dense deposits within or in the subendothelial location of GBM, and the presence of platelet aggregates and fibrin (or platelet aggregates alone) within the lumen of the glomerular capillaries (Figs. 9-

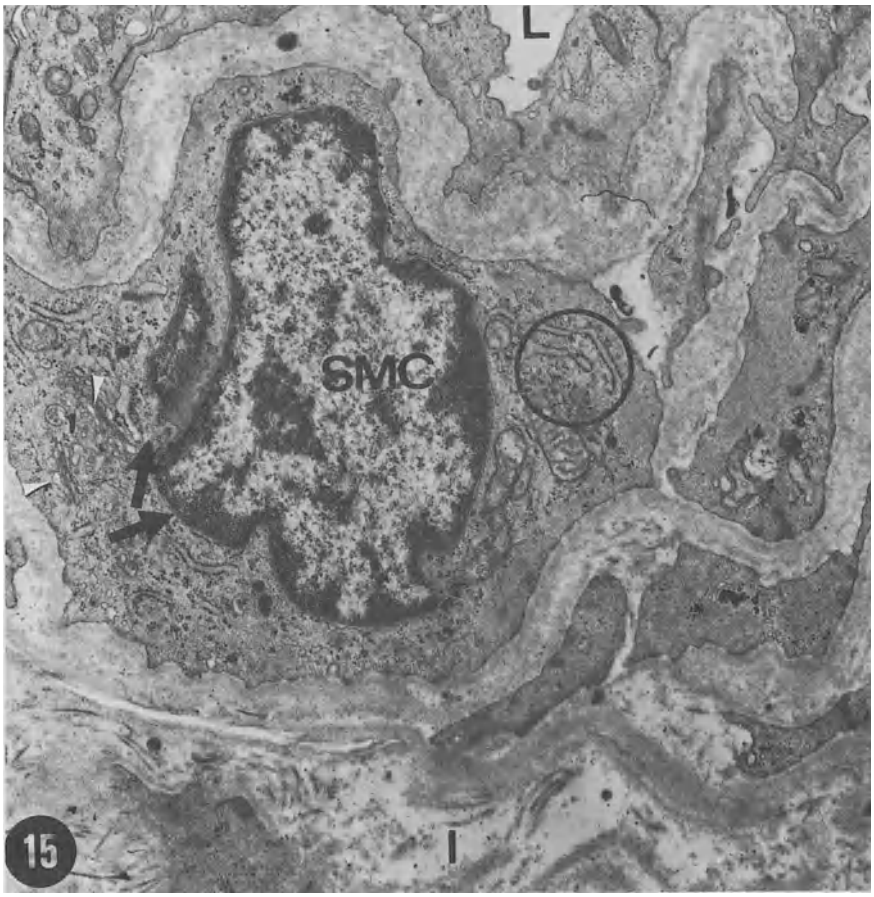

Fig. 9-15. Discernible hyperplasia of smooth muscle cell (SMC) characterized by double nuclei (arrows), many rough-surfaced endoplasmic reticula (circle), and prominent Golgi complexes (arrowheads). Lumen of the arteriole (L) is shown. Outer border merges with the interstitium (I) (UA + LC, ×15,000). From the same specimen as Fig. 9-11.

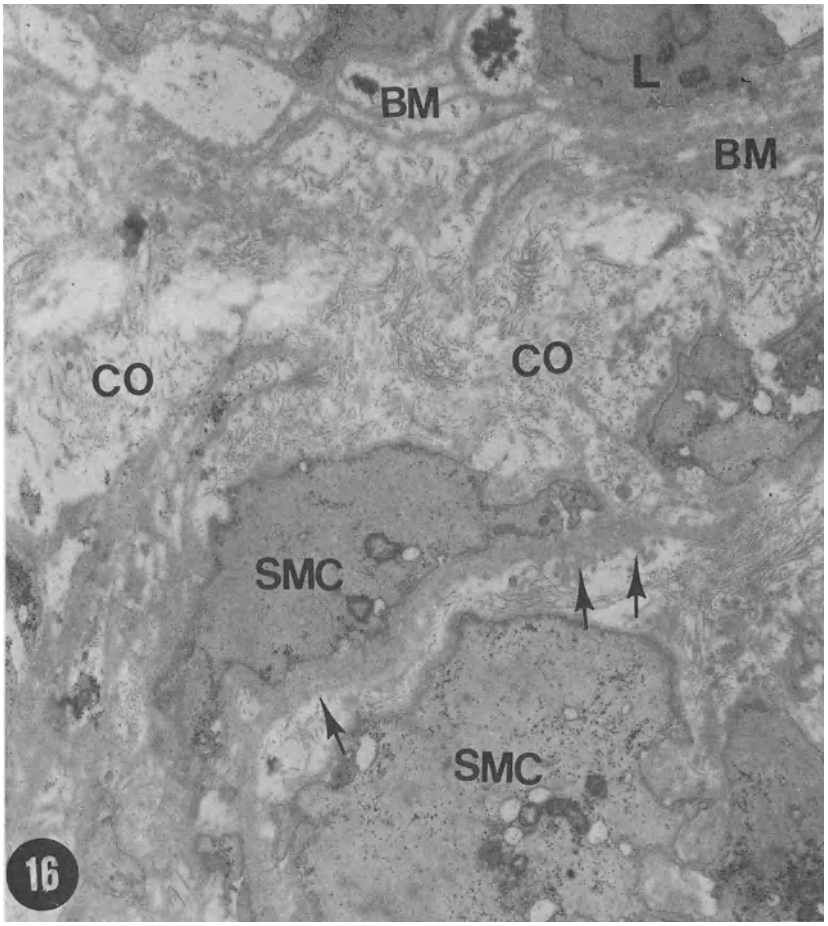

Fig. 9-16. In this necrotic arteriole, note fibrillar basement membrane (BM). Necrotic smooth muscle cells (SMC) are characterized by absent or few cell organelles, absence of membrane, electron-dense basement membrane between SMC (arrows), and excessive amounts of collagen fibers (CO) surrounding the SMC. Lymphocyte (L) within the lumen is shown (PAMS, ×12,000). From the same specimen as Fig. 9-11.

31 to 9-33). Tubules are essentially normal and no discernible change has been observed in the interstitium.

Immunofluorescence Microscopy Study in Rats

The fluorescence for fibrinogen was most marked in the glomeruli of the old SHR.* Among the untreated old SHR, IgG was 1+ to 2+ commonly and 3+ to 4+ rarely in 25 to 50% of the glomeruli in two-thirds of the rats (Fig. 9-36), and

* The intensity of fluorescence was scored on a 0 to 4+ scale: 0 = negative, 1+ = trace, 2+ = mild, 3+ = moderate, 4+ = intense.

negative in one-third of the rats; IgG was negative to 1+ in the vessels; fibrinogen was 3+ to 4+ in 50 to 75% of the glomeruli in three-fourths of the total rats (Fig. 9-37) and negative in one-fourth of the rats; fibrinogen was 3+ to 4+ in vessels with arteritis only, and negative to 1+ in intact vessels; γG was 1+ to 4+ in approximately 50% of the glomeruli in one-half of the rats, and negative in normal vessels; and C3 was 1+ to 2+ in about 50% of the glomeruli in five rats studied. C3 was negative in all vessels.

Among the heparin-treated old SHR, IgG in the glomeruli and vessel was not appreciably different from that in the glomeruli and vessel of untreated old SHR; fibrinogen in the glomeruli was markedly decreased, having 1+ to 2+ in most glomeruli (Fig. 9-38) and negative to 1+ in the vessels. γG and C3 were not

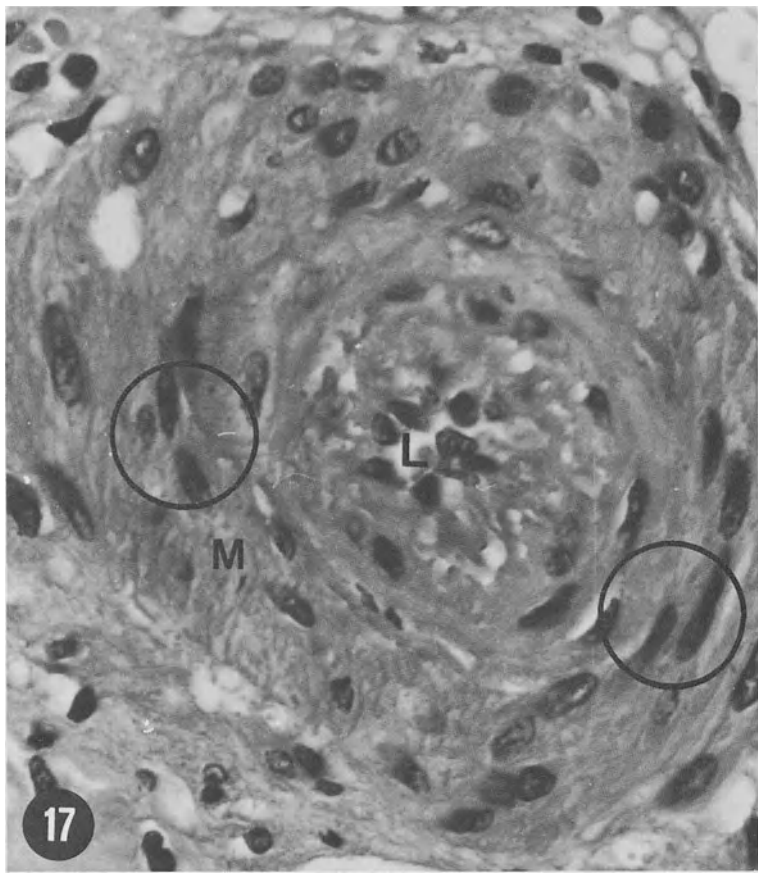

Fig. 9-17. Hypercellular arteriole with hyperplasia involving mostly smooth muscle cells in the media (M). Some SMC reveal hyperchromatic nuclei (circles). The inner aspect of the arteriole shows dark matrix with ooclusion of the lumen (L) (H & E, ×320). From the renal biopsy of patient #8 in Table 9-1.

appreciably different than those in untreated old SHR. In all young SHR and in WKY of all ages, IFM was very unimpressive.

Heparin-treated SHR also showed significant decreases in histopathological scores. In these rats, arterial blood pressures were also remarkably low. The difference in MAP between heparin-treated old SHR and untreated old SHR was found to be significant.

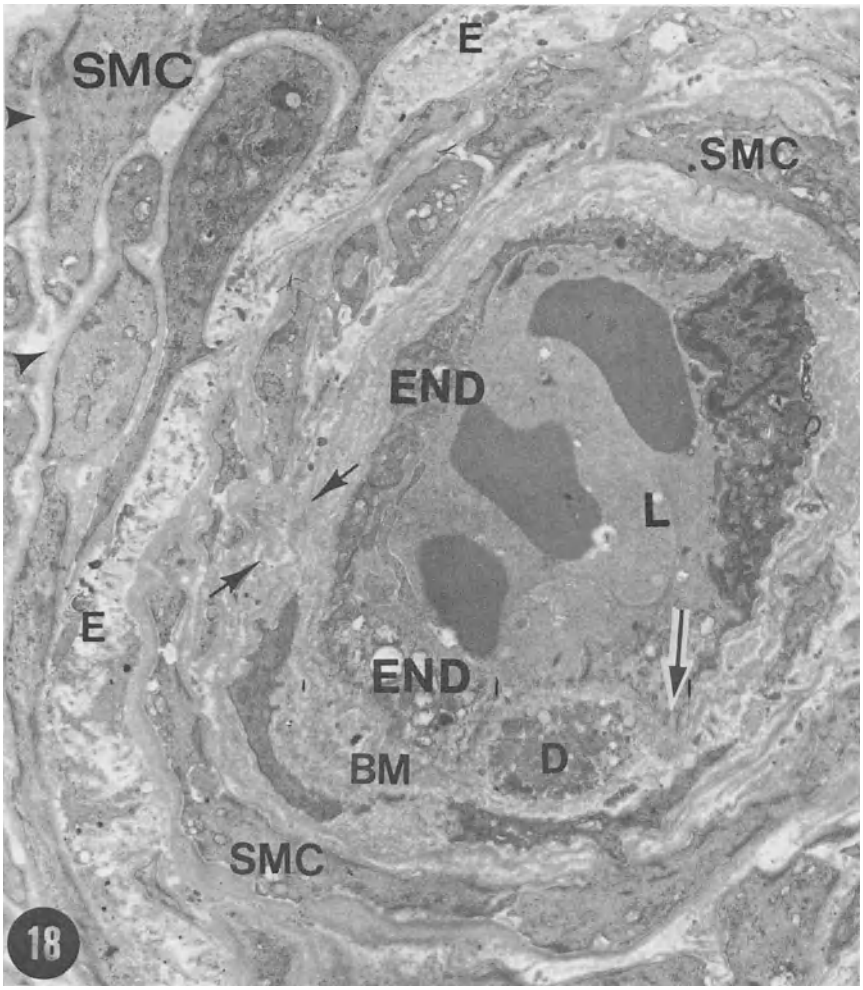

Fig. 9-18. This large arteriole (or small artery) demonstrates a variety of changes: (1) Necrotic and fibrillar basement membrane (BM); (2) continuity between the lumen (L) of the arteriole and the BM (black and white arrow); (3) completely necrotic inner smooth muscle cells (SMC); (4) continuity between BM and SMC layers (dark arrows); (5) edema and necrotic debris (E) between layers of SMC; (6) partially necrotic outer smooth cells (SMC); (7) an electron-dense deposit (D); (8) partially disrupted endothelial cells (END); (9) necrotic outer border of SMC (black arrowheads) (UA + LC, ×15,000). From the same specimen as Fig. 9-17.

The relationship of renal lesions to mean arterial blood pressure and age was analyzed statistically in all three strains of rats. For the NR and WKY a nonsignificant inverse relationship between the index scores of renal morphology and MAP was found. A significant direct relationship was observed between index scores and MAP in SHR (correlation coefficient r of 0.43, $p<0.05$). This positive correlation was attributable to the slightly higher blood pressures observed in the old SHR, who uniformly had high index scores (Fig. 9-39). Since age was not a controlled variable in the collection of data and since index scores and blood pressure may be related to age, frequency distributions for age versus index

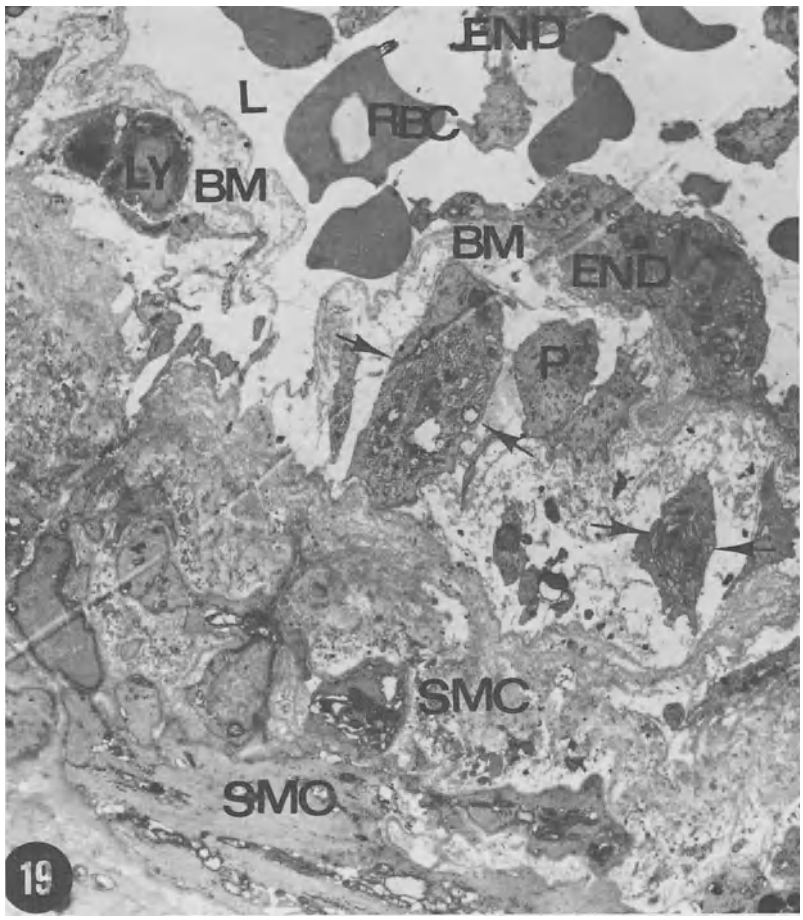

Fig. 9-19. This arteriole (or small artery) exhibits total necrosis characterized by (1) detachment of the endothelial cells (END) and shedding off into the lumen (L); (2) necrotic, edematous, and fibrillar basement membrane (BM); (3) infiltration of the wide subendothelial space by lymphocyte (LY), plasma cells (pointing arrows), platelet (P), and widespread necrosis of smooth muscle cells (SMC); (4) an inclusion body within the red blood cell (RBC) (UA + LC, ×12,000). From the same specimen as Fig. 9-17.

scores were prepared. For the NR, a small negative nonsignificant slope and correlation coefficient were found. For the WKY, a positive slope and significant correlation coefficient ($r = 0.40$, $p < 0.05$) were found. For the SHR, a highly significant positive slope and correlation coefficient ($r = 0.742$, $p < 0.01$) were obtained (Fig. 9-40). This finding applies solely to the high index scores observed in the old SHR with mean age of 91 weeks. No linear relationship was found to exist for young SHR (52 weeks), and no linear relationship between index scores and age was observed in any animal under 30 weeks of age. Since SHR has

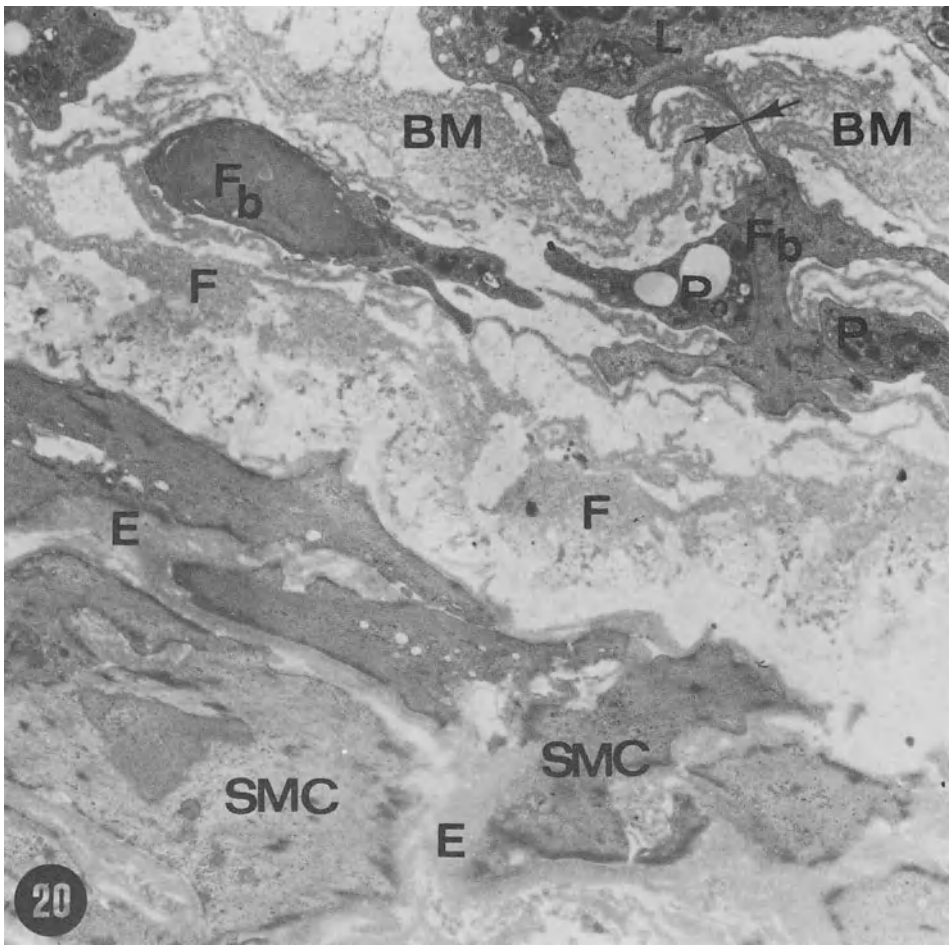

Fig. 9-20. In this arteriole necrotic and infiltrative changes are discerned. Note (1) a lymphocyte (L) in the luminal side of the arteriole; (2) necrotic and fibrillar basement membrane (BM); (3) intrusion of a process (opposing arrows) of a fibroblast (Fb) through BM; (4) infiltration of platelets (P), fibroblasts (Fb), and (5) fibrinlike materials (F); (6) widespread necrosis of smooth muscle cells (SMC); and (7) edema (E) between necrotic SMC (UA + LC, ×18,000). From the same specimen as Fig. 9-17.

elevated arterial blood pressure from the earliest age at which pressure can be measured (4 weeks), age in SHR can be equated with the duration of hypertension. The highly significant association between age (i.e., duration of hypertension) and severity of renal lesions in SHR, therefore, provides evidence to support a concept that the severity of renal lesions is more dependent on the duration than the severity of hypertension.

RATIONALE OF STUDIES OF HYPERTENSIVE ANIMALS

Experimentally Induced Hypertension

Numerous investigators have studied renal and extrarenal vascular lesions in rats, rabbits, and dogs after producing hypertension by unilateral or bilateral

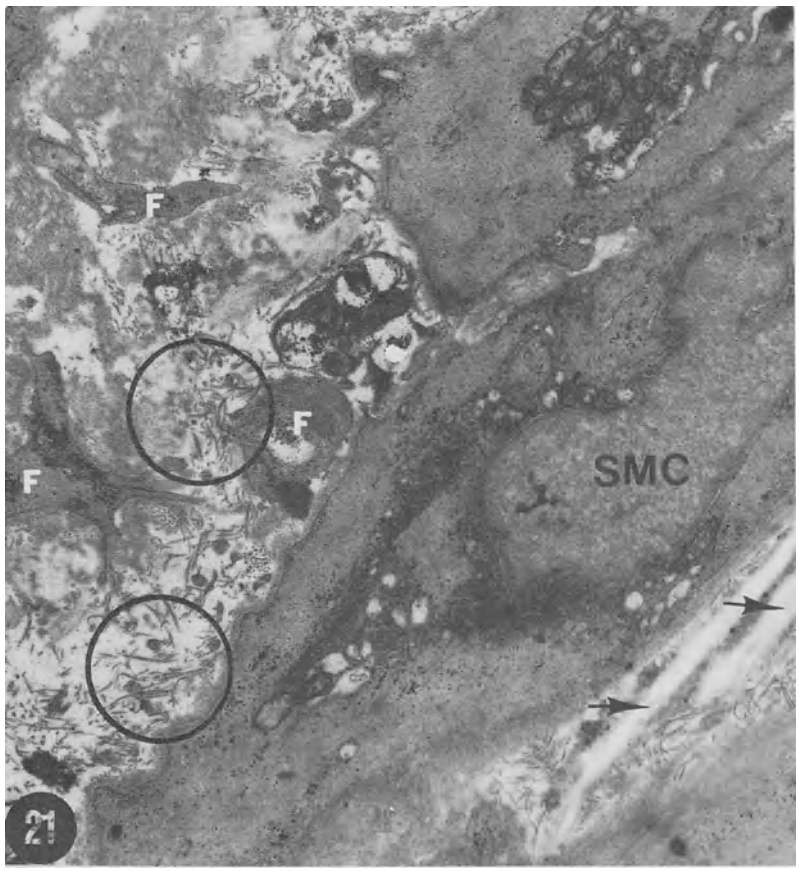

Fig. 9-21. Infiltration by excessive amounts of collagen fibers (circles) and many fibroblasts (F) between and around necrotic smooth muscle cells (SMC) are clearly discerned. Nonstained elastic tissue (arrows) is shown (PAMS, ×18,000). From the same specimen as Fig. 9-17.

constriction of renal artery, by unilateral constriction of the renal artery with removal of the contralateral kidney, by silk wrapping of both kidneys, and so on (Table 9-8). The maximum period of observation in the experimentally produced hypertensive animals was 24 weeks. Serial systolic blood pressure was measured in all experiments and hypertension was documented. In all the experimental studies (Table 9-8), there had been a unanimity in the authors' observations, i.e., necrotic renal and extrarenal vascular lesions along with arteritis in some of the experimental groups. Almost all the authors concluded that these vascular lesions were caused by greatly increased arterial pressure.

Even though these experimental studies contributed to our understanding of the relationships between renal lesions and hypertension, there are still many

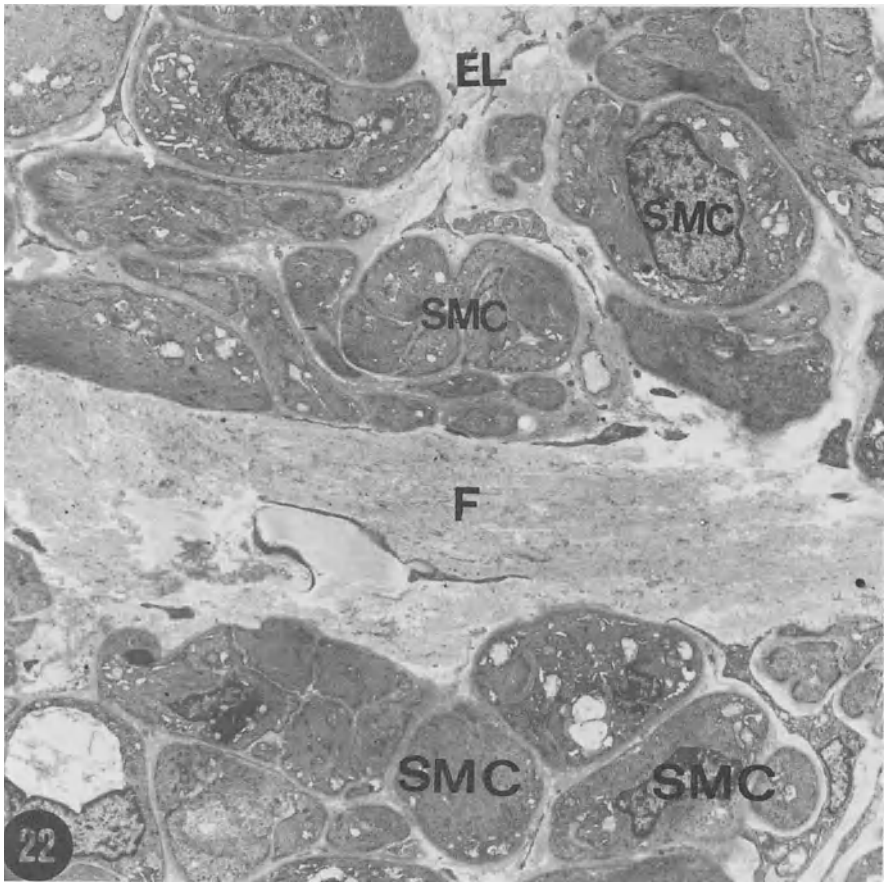

Fig. 9-22. This figure demonstrates (1) hyperplasia of smooth muscle cells (SMC) forming concentric bundles, (2) a band of fibrin (F) separating the SMC bundles, and (3) the electron-lucent areas (EL) between the SMC bundles representing edema and/or elastic tissue (UA + LC, ×16,000). From the same specimen as Fig. 9-17.

questions which must be answered in order to apply the analogy of the experimental hypertension model to essential hypertension in the human. The difficulties and the questions relative to these experimental models are as follows:

1. Although in the previous studies vascular lesions had been stated to be necrotic, rarely was this finding confirmed by EM study. The only exception is the study of Goldby and Beilin (1974).

2. Although all the previous studies were well designed and well controlled, the histological observations were arbitrary and the findings were not subjected to critical analysis.

3. Wilson and Byrom (1939) thought duration of hypertension could be a factor in the pathogenesis of renal lesion but never confirmed that relationship.

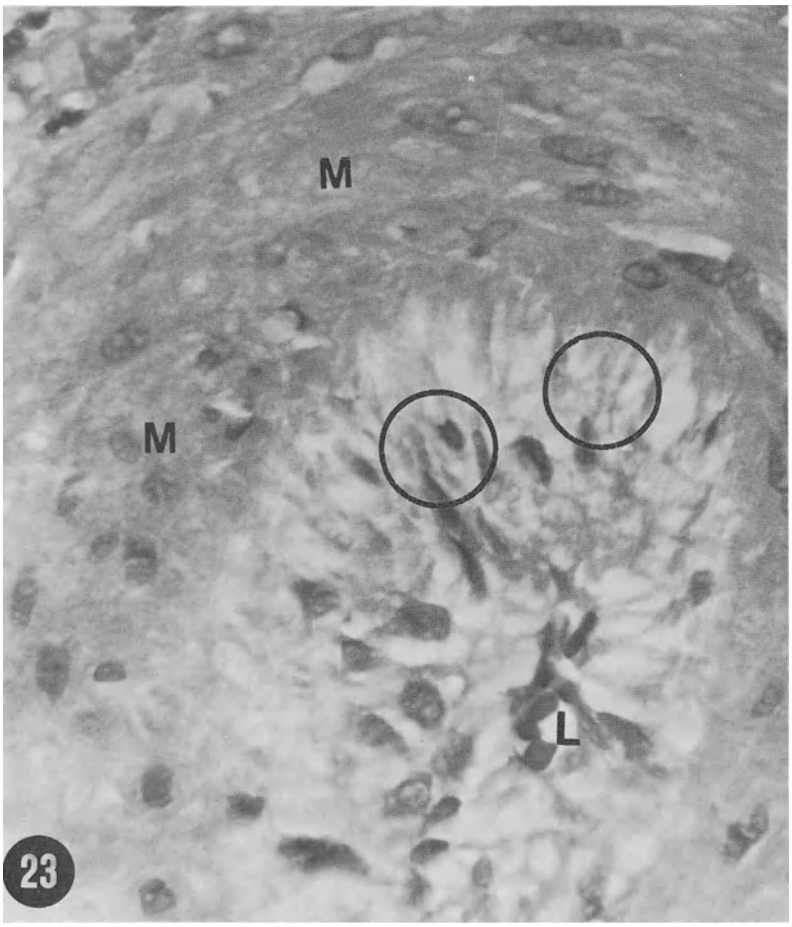

Fig. 9-23. Excessive proliferation of fibroblasts (circles) in a large arteriole with complete occlusion of the lumen (L), often described as fibroplasia. Proliferation of the smooth muscles and thickening of media (M) are also seen (H & E, ×320). From the renal biopsy of patient #10 in Table 9-1.

4. Plasma renin activity seldom, if ever, was measured in the experimentally induced hypertensive animals.

Therefore, it seems clear that experimentally induced hypertensive models are not ideal models with which to study essential hypertension in humans. This conclusion is supported further by the fact that necrotic lesions are very uncommon in the contralateral kidney or extrarenal arterial vascular beds in renovascular hypertensive patients. Even so, in humans, renovascular hypertension differs greatly from essential hypertension.

Spontaneously Hypertensive Rat

The histological studies of kidneys in SHR of all ages compared with those in normotensive control rats have been completed in order to evaluate the relative

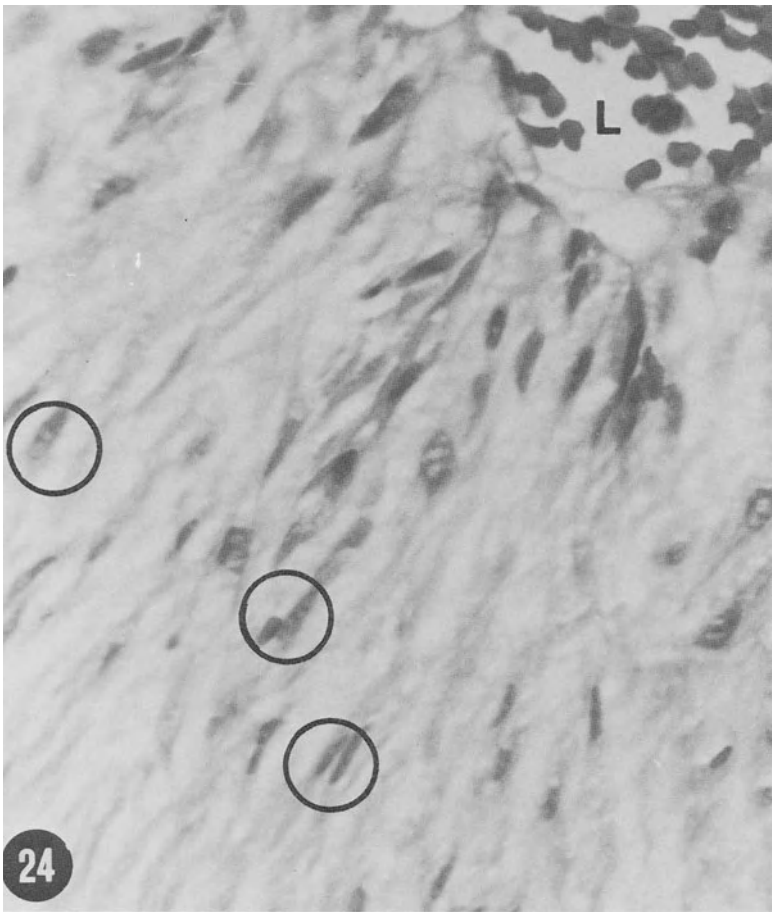

Fig. 9-24. Magnified view of the arteriole seen in Fig. 9-23 showing elegant appearance of proliferation of fibroblasts (circles). In the lumen of the arteriole (L), accumulation of RBC is seen (H & E, ×800).

importance of severity of hypertension and age in hypertensive species (i.e., duration of hypertension) in the development of two major types of renal vascular lesions commonly associated with essential hypertension in humans. The first lesion (grade II or III lesion), similar to that seen in benign essential hypertension in humans, is characterized by thickening of the arteriolar basement membranes, excessive BM-like material throughout the vascular wall, and normal or atrophic SMC. The second lesion (grade IV), similar to that seen in malignant hypertension in humans, is characterized by polyarteritis, proliferative or necrotic changes in the large or small arterioles, glomerular necrosis and/or thrombosis, tubular atrophy, and interstitial fibrosis. However, some early lesions, showing hyper-

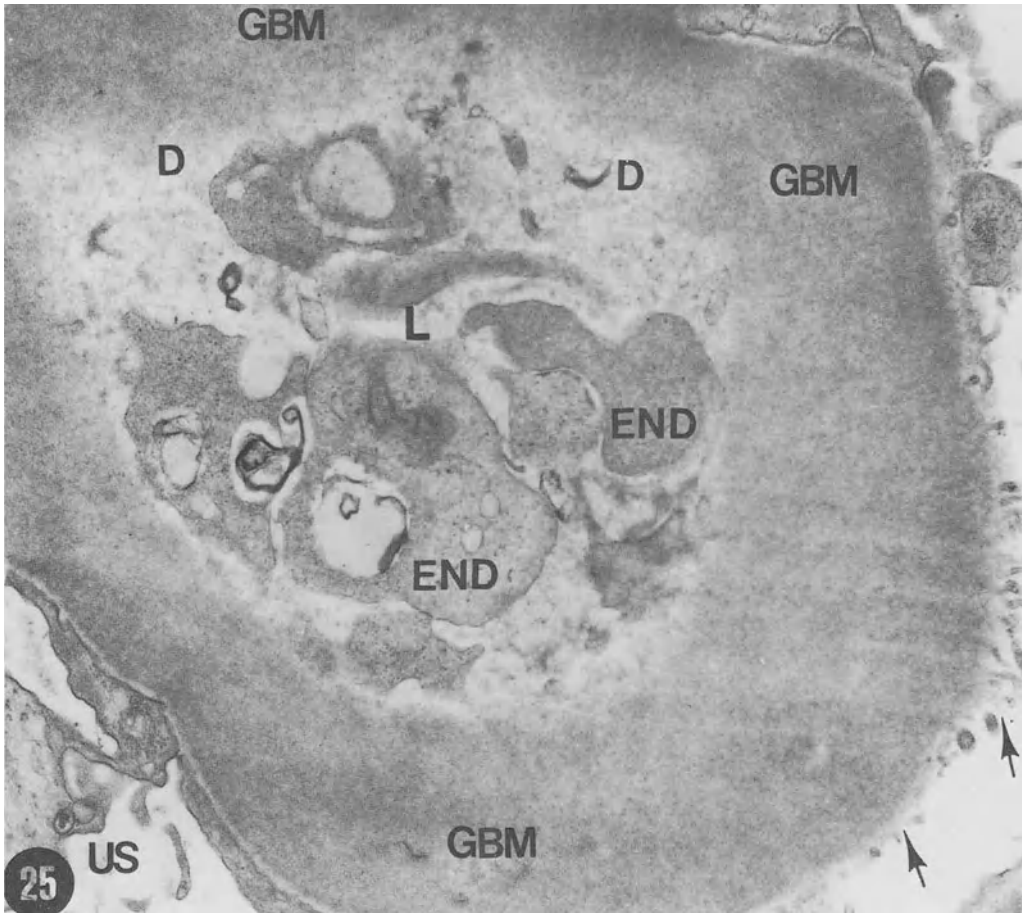

Fig. 9-25. A glomerular capillary shows (1) extremely electron-dense basement membrane (GBM), (2) necrosis of foot processes (arrows), (3) necrosis and denudation of endothelial cells (END), and (4) electron-lucent areas reminiscent of electron-dense deposits (D). Hardly discernible lumen (L). Urinary space (US) is shown (UA + LC, ×21,000).

plasia of SMC rather than the atrophy of SMC observed by EM in the setting of more benign lesions, were retrospectively found in SHR and not in WKY or NR. It would appear then that the early vascular changes of malignant hypertension, though not separable from those observed only in benign hypertension by LM study and our scoring system (grade II and III lesions), are distinctly different as shown by the use of electron microscopy. Thus, this analysis affirms the pertinence of the spontaneously hypertensive rat model in studying the relationship between hypertension and renal lesions.

RELATIONSHIP BETWEEN RENAL LESIONS AND HYPERTENSION

Question

Observations in experimentally induced hypertensive models suggest that renal lesions are caused by hypertension. This is claimed on the basis of the

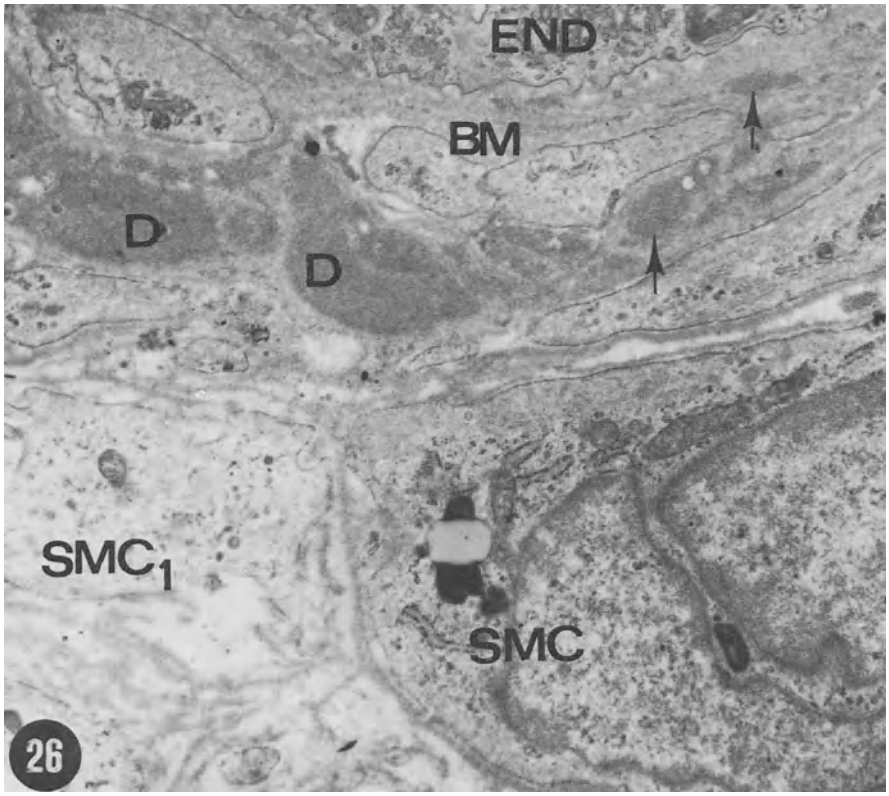

Fig. 9-26. In this arteriole (1) conspicuous electron-dense deposits (D and arrows) within and under the basement membrane (BM) of the endothelial cell (END), (2) normal smooth muscle cell (SMC), and (3) necrosis of other smooth muscle cells (SMC_1) can be seen (UA + LC, ×18,000). From the renal biopsy of patient #7 in Table 9-1.

Table 9-5
Scoring (LM) of Renal Lesions in Hypertension

Histological changes in all component parts of the kidney	Score
A. Artery and arteriole	
1. No change observed	0
2. PAS-positive matrix	
a. Minimal (+) PAS-positive matrix in the intima only	1
b. Much (++) PAS-positive matrix throughout the vessel wall	2
3. Vacuoles	
a. Few vacuoles in media only	1
b. Many vacuoles throughout the vessel wall	2
4. Hyperplasia of smooth muscle cells	
a. Without occlusion of the lumen	3
b. With occlusion of the lumen	5
5. Necrotic changes	
a. Intense PAS-positive homogeneous material in the intima	5
b. Intense PAS-positive homogeneous material throughout the vessel wall	5
c. Infiltration of RBC and inflammatory cells within and outside the vascular wall	5
B. Glomeruli	
a. Increase in mesangial matrix	1
b. Intracapillary thrombi	2
c. Necrosis of tufts	2
d. Crescents (greater than 10%)	2
C. Tubules and interstitium	
a. Atrophy and dilatation of tubules	2
b. Interstitial infiltrates or fibrosis	1
Total points	39

Source: From Mandal *et al.* (1977*a*,*c*), by the kind permission of the editors.

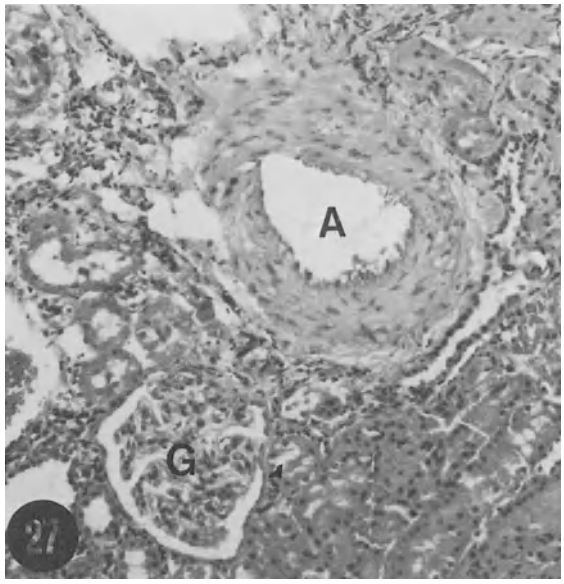

Fig. 9-27. This figure shows grade II lesion characterized by hyperplasia of smooth muscle cells of arteriole (A) without compromise of the lumen. A normal glomerulus (G) is seen (H & E, ×80).

absence of renal lesion in normotensive control animals. In all probability it is true for experimentally induced hypertensive animals, but it is unclear as to what extent this is applicable to renal lesions found in association with human essential hypertension. Necrotic renal lesions are infrequent in patients presenting with severe hypertension. This has been illustrated in patient #5. It is reasonable to state that in essential hypertension, it is still unclear whether hypertension causes the renal lesions or vice versa. There are many problems which stand in the way of answering this question about humans, including: (1) It is difficult to obtain a serial renal biopsy or any renal biopsy in asymptomatic hypertensive patients.

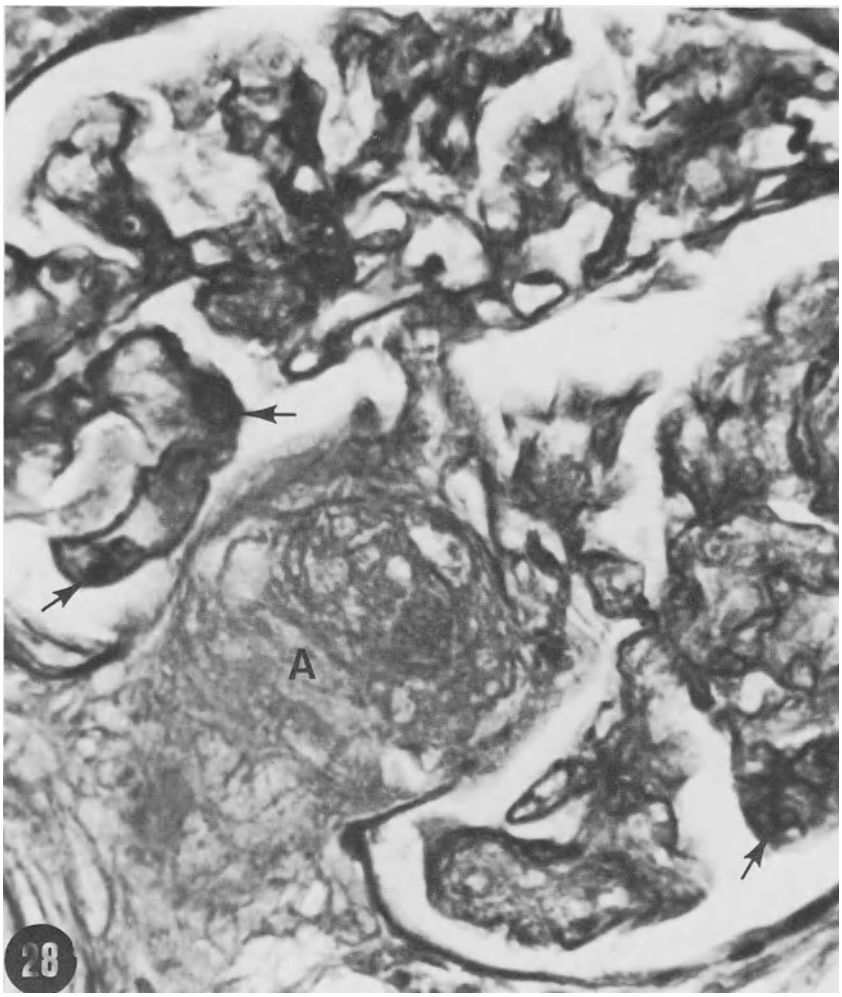

Fig. 9-28. (1) Necrosis of glomerular arteriole (A) (afferent or efferent) but intact glomerular capillaries; (2) diffuse irregular thickening of basement membrane of peripheral capillary loops (arrows) (PAS, ×320).

(2) Single biopsies are performed randomly in hypertensive patients, but they are simply inadequate to provide an understanding of the sequence of development of the renal lesions. (3) Biopsies obtained percutaneously are often inadequate for study of blood vessels, and a considerable sampling error for the larger vessels (small arteries and arterioles) may exist. (4) Specimens obtained from autopsy show some degree of autolysis and are less than optimum for light microscopic examination and inadequate for electron microscopic examination. (5) Serial biopsies from kidneys or normotensive subjects also are difficult to obtain, so

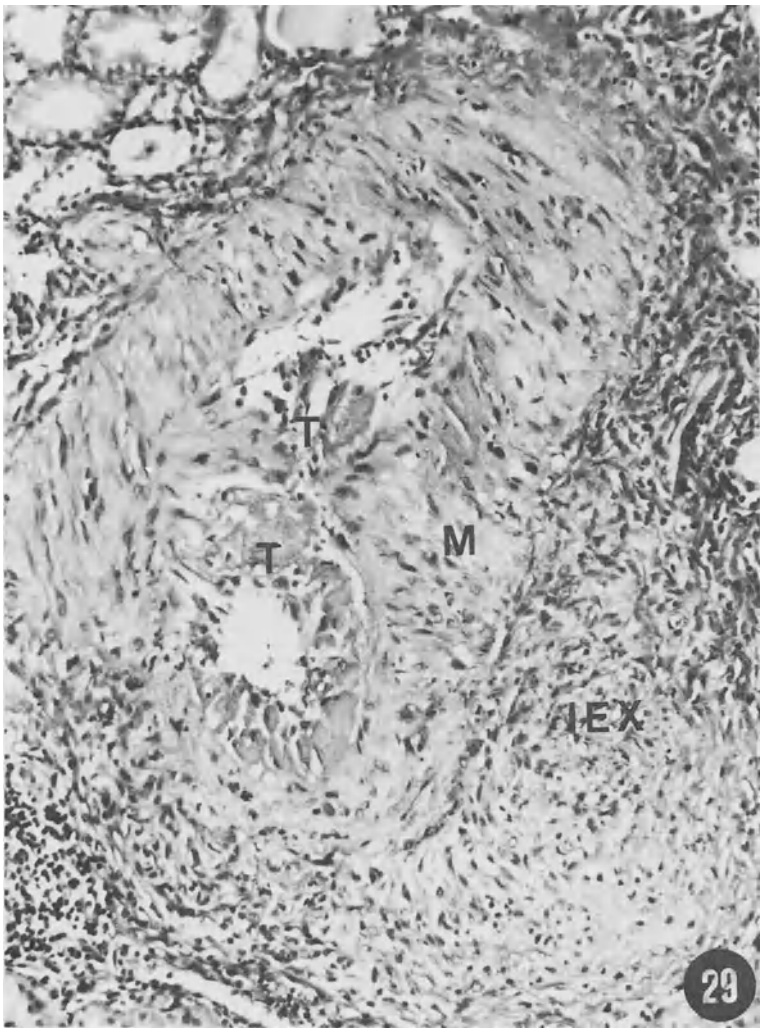

Fig. 9-29. Arteritis in this small artery shows (1) diffuse necrosis with thrombosis (T) in the inner aspect, (2) necrosis of a part of the medial layer (M), and (3) inflammatory exudate (IEX) surrounding the right half of the necrotic artery (H & E, ×320).

the effect of aging on vessel pathology cannot be separated from the effect of the hypertension. (6) Interpretation of renal morphology is often biased by a knowledge of the clinical information available on the patients. (7) The lack of a universal agreement on the definition of clinical benign and malignant hypertension has complicated further evaluation of the renal pathology in hypertension.

At this time, one might also question the appropriateness of the terminology *nephrosclerosis* as used to define the renal lesions in essential hypertension. In Stedman's *Medical Dictionary* (18th edition), nephrosclerosis is defined as "induration of the kidney from overgrowth and contraction of the interstitial connective tissue." Are we then justified in using this general term to describe the renal lesions in essential hypertension? A component of overgrowth is seen in the arterioles either as excessive thickening of basement membrane or BM-like materials or as hyperplasia of smooth muscle cell. Fibrosis of the interstitium is not a pronounced feature, at least not enough to cause shrinkage of the kidney,

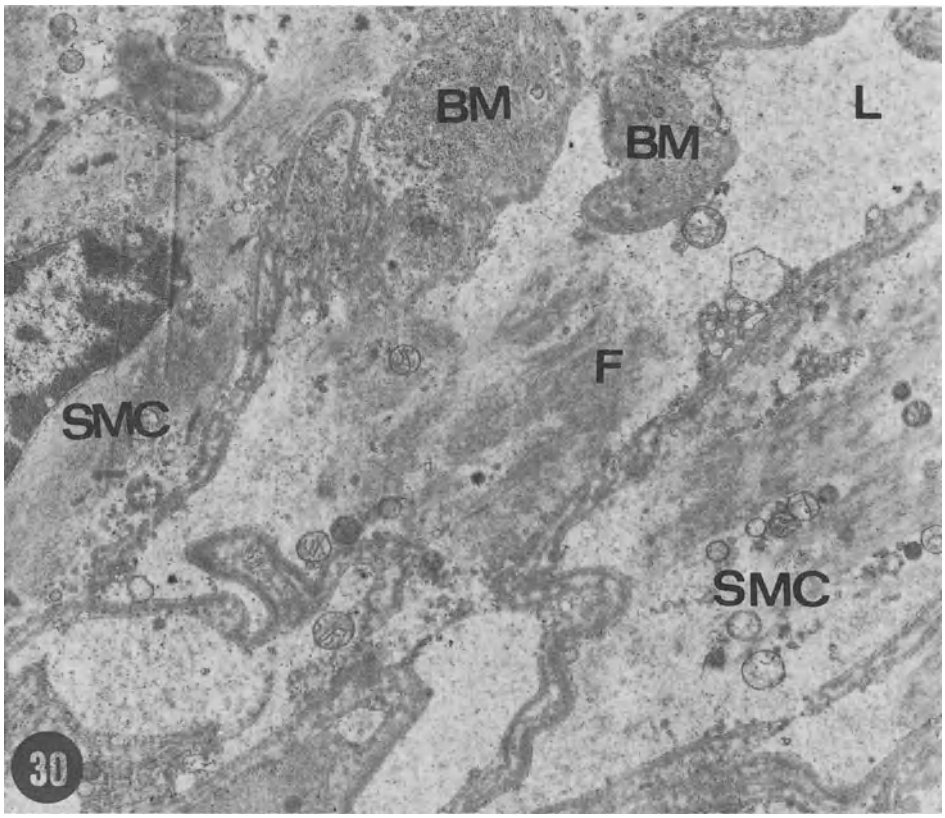

Fig. 9-30. EM of a large arteriole reveals diffuse necrosis of basement membrane (BM), smooth muscle cells (SMC), and fibrinlike fibers (F). Lumen of the arteriole (L) can be seen (UA + LC, ×18,000).

regardless of the clinical severity of hypertension. In benign hypertension, there is a modest reduction in the size of the kidneys, the total weight being over 200 g (normal total weight of two kidneys is 250–300 g). In malignant hypertension, the combined weight varied from 130 to 410 g in one series and from 180 to 380 g in another series. Hyalinization of glomeruli and atrophy of tubular cells can cause reduction in weight. Therefore "nephrosclerosis" is at the least not a satisfactory term to denote the microscopic picture of the kidney in hypertension; however, it seems appropriate to use the terms *benign* and *malignant* to indicate mild and severe lesions, respectively.

Since all the observations of renal pathology in essential hypertension have been retrospective, let us resume our discussion relating the renal pathology to

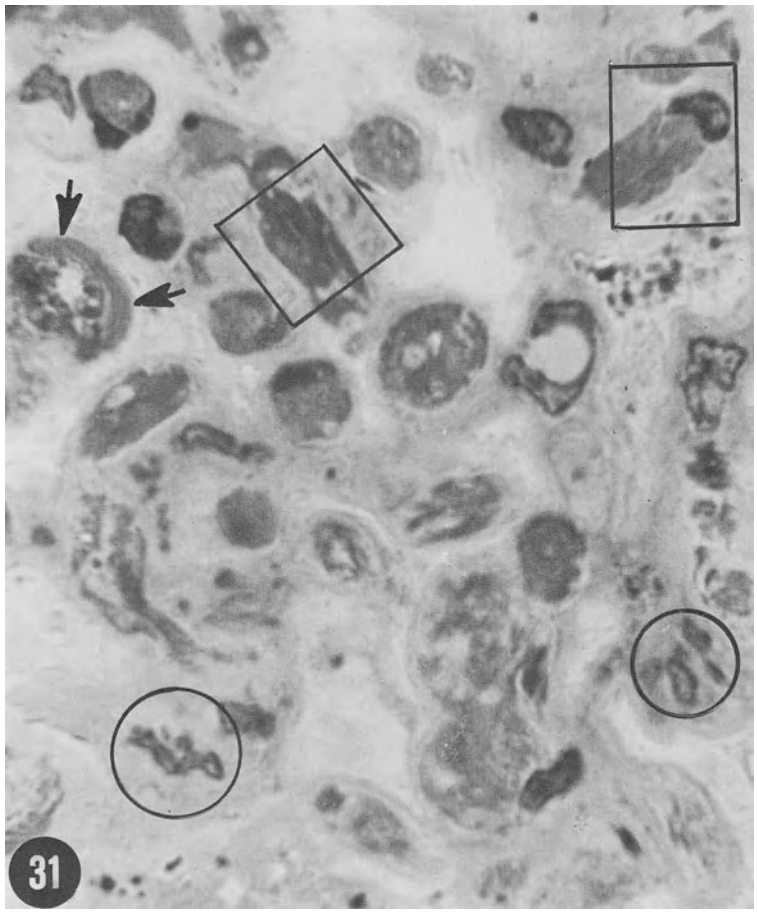

Fig. 9-31. In the survey section of a glomerulus fibrin accumulation within glomerular capillaries (squares), exudation of neutrophil leukocytes (circles), and deposit within a capillary (arrows) are shown (methylene blue-azure II, ×800).

clinical benign and malignant hypertension. Kincaid-Smith and colleagues (1958), based on their own studies as well as a review of studies of other authors, consider that papilledema is the hallmark of malignant hypertension, a definition that seems to be acceptable to most individuals. Using this definition, we will attempt to define some of the relationships of the renal lesions to essential hypertension. In this series, patients 1 through 6 appear to have benign hypertension. The renal histopathology is similar in the first four patients, whereas the renal histopathology, especially by EM, in patients 5 and 6 resembles that observed in patients 7 through 10 (Tables 9-3 and 9-4). The LM studies in the first four patients are characteristic of arteriolar (i.e., benign) nephrosclerosis: Ultrastructurally, the changes consist of a thickening of basement membrane, exces-

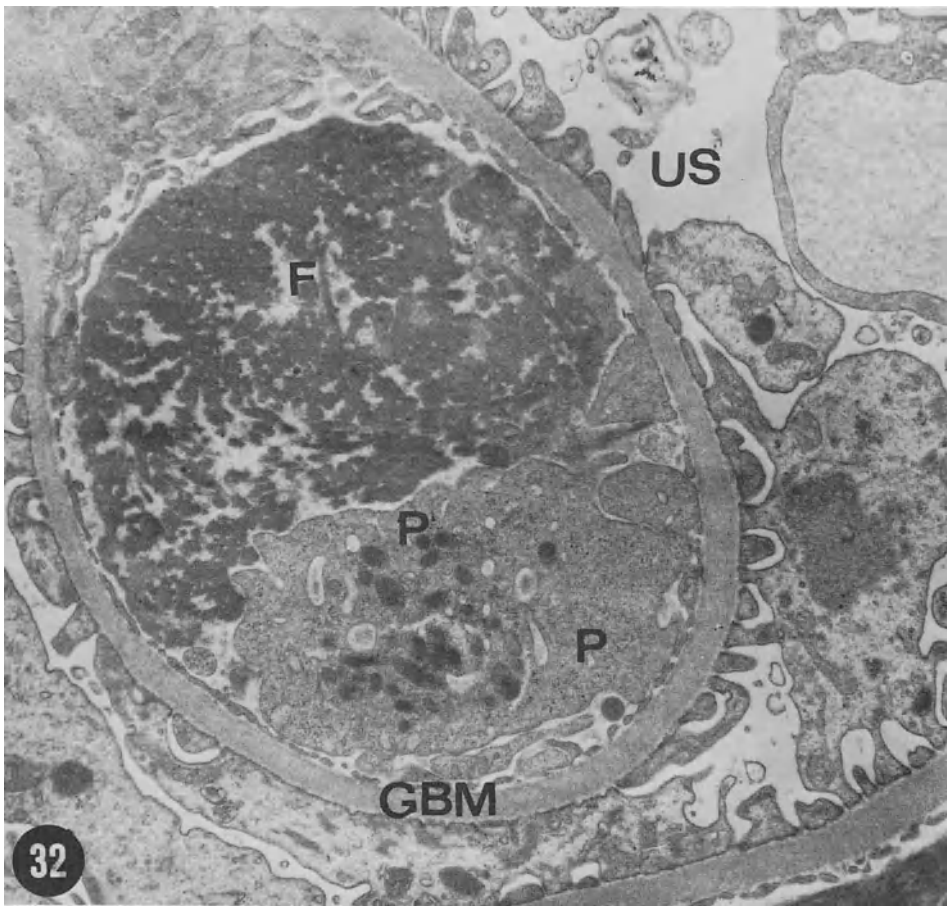

Fig. 9-32. Widespread thrombosis in the glomerular capillaries: (1) massive accumulation of fibrin (F); (2) platelet aggregates (P)—some platelets are depleted of granules while others have a few granules; (3) reasonably normal glomerular basement membrane (GBM). Urinary space (US) is shown (UA + LC, ×19,000).

sive amounts of BM-like material, and atrophy of smooth muscle cells. McGee and Ashworth (1963), as well as Biava and associates (1964), found similar ultrastructural arteriolar changes in benign or essential hypertensive patients. These changes appear to be nonspecific since they can be observed in the renal arterioles in chronic renal disease without hypertension (Mandal *et al.*, 1978*a*) and in old age. These observations suggest the lack of any specific relationship between clinical benign hypertension and the renal pathology found in these patients. In other words, benign hypertension and arteriolar (i.e., benign) nephrosclerosis may be independent variables. This is supported by the lack of relationship between grade II and III renal lesions and the severity of mean arterial pressure in SHR (Fig. 9-39).

Fisher and Pirog (1976) produced contracted kidneys by selective partial

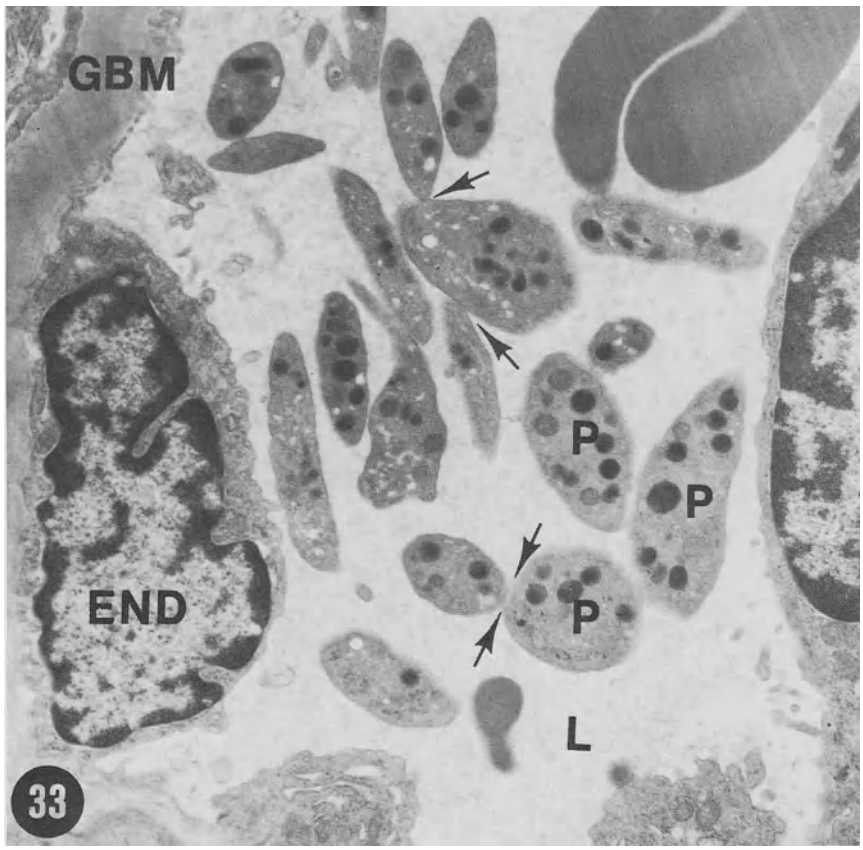

Fig. 9-33. Massive accumulation of platelets within the lumen (L) of the glomerular capillary. The platelets show (1) loss of membrane in most (P), (2) fusion between some (arrows), (3) electron-dense glomerular basement membrane (GBM). Endothelial cell (END) is shown (UA + LC, ×18,000)

occlusion of the intrarenal arteriolar bed with microspheres in Sprague-Dawley rats without elevation of blood pressure. LM study of the kidney revealed lesions consistent with arteriolar (i.e., benign) nephrosclerosis, which led these authors to conclude that such arteriolar lesions are pathogenetically unrelated to hypertension.

In contrast, a direct relationship seems to exist between hypertension and the renal lesions characterized by proliferative and necrotic changes in the arterial vessels associated with hyalinization, necrosis and thrombosis of the glomeruli, profound disappearance of tubules, and moderate to marked interstitial changes. This hypothesis is supported by data emerging from several large clinicopatho-

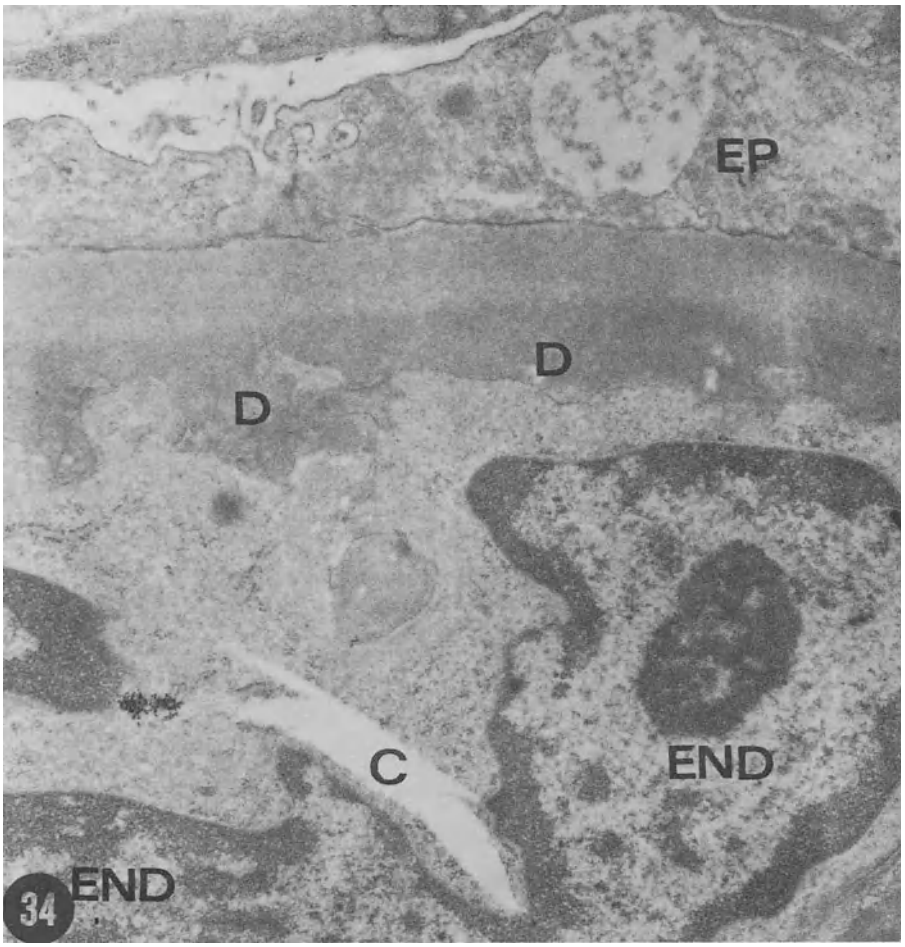

Fig. 9-34. This glomerular capillary demonstrates conspicuous electron-dense deposit in the subendothelial location (D). Note proliferation of endothelial cell (END) and fusion of foot processes and epithelial cell (EP). A cholesterol crystallike structure (C) appears to be an artifact (UA + LC, ×21,000).

Table 9-6
Grading of Renal Morphological Changes in Order of Increasing Severity

Grade	Description	Score	Severity
I	No change, minimal PAS-positive material in intima, and/or a few vacuoles in media in small artery or arteriole	0–2	Normotensive
II	Much PAS-positive material throughout and few vacuoles in media or both throughout, smooth muscle cell (SMC) hyperplasia without narrowing of lumen (small artery or arteriole), no tubulointerstitial change	3–7	Mild hypertension
III	Any of the grade II combinations plus narrowing of arteriolar lumen plus increased glomerular matrix and/or glomerular crescents	8–19	Moderate hypertension
IV	Arteriolar SMC hyperplasia with luminal narrowing, periarteritis, glomerulitis, necrosis in the arterioles and glomeruli, glomerular thrombosis, glomerular crescents (more than 10%), tubular atrophy and dilatation, and interstitial change (more than 10%)	20–39	Severe (or malignant) hypertension

Source: From Mandal *et al.* (1977*a*,*c*) by the kind permission of the editors.

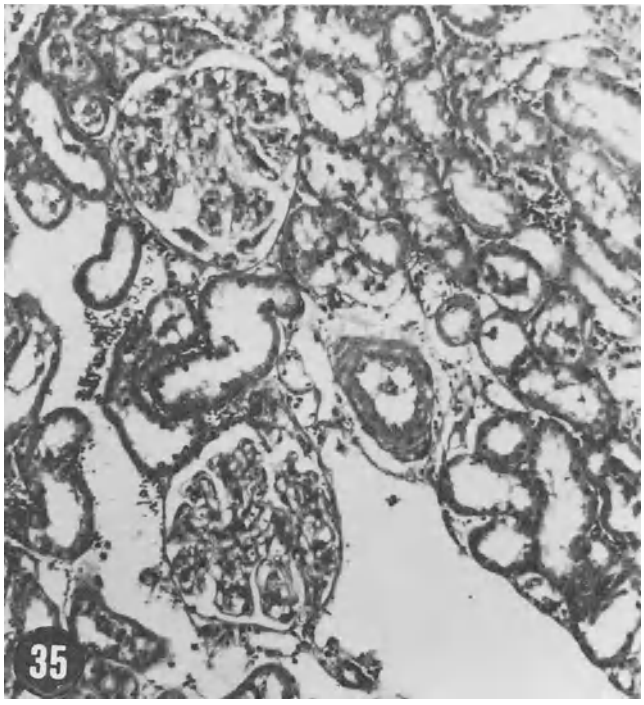

Fig. 9-35. Normal appearance of all the component parts of the kidney, i.e., glomeruli, tubules, arteriole, and the interstitium (H & E, ×80).

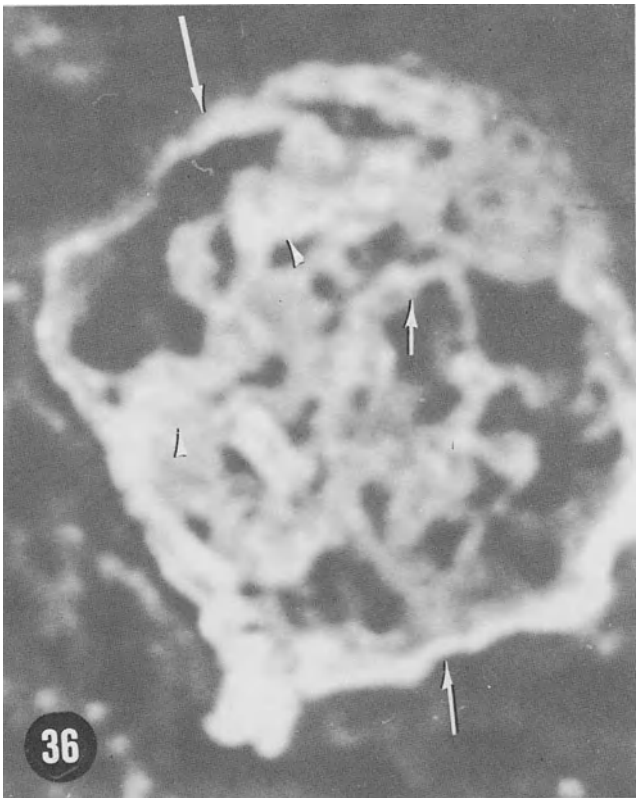

Fig. 9-36. In the glomeruli is seen 3+ IgG in the peripheral capillary loops (short arrow), mesangium (arrowheads), and the Bowman's capsule (long arrows) (IFM, ×1600). From a 112-week-old untreated SHR.

logical studies in malignant hypertension. Before we review these clinicopathological correlations, let us resolve some of the problems which tend to muddle the understanding of the relationship. Controversy exists over which of the two arteriolar lesions, endarteritis proliferans (proliferation of SMC and intimal fibroplasia with occlusion of the lumen) or necrotizing arteriolitis (arteriolar necrosis),

Table 9-7
Distribution of All Rats According to Grades of Severity of Renal Histopathology

		Grades of renal histopathology (% of total)			
Rats	N^a	I	II	III	IV
SHR	46	17	54	9	20
NR	35	83	17	0	0
WKY	28	64	32	4	0

[a] Number of rats.

is the principal lesion in malignant nephrosclerosis. This issue continues to intrigue the pathologist and practicing physican. A review of the literature reveals a lack of agreement on this question. Kimmelstiel and Wilson (1936) and Kincaid-Smith (1975) considered diffuse proliferative endarteritis as the characteristic histological lesion of malignant hypertension, whereas Heptinstall (1953, 1974) regarded arteriolar necrosis as the hallmark of malignant hypertension. Proliferation of smooth muscle cells is thought to be a major factor in causing occlusion of the vascular lumen (Spiro *et al.*, 1965). However, when an arteriole with proliferative changes (LM) is examined by EM, it almost always reveals fibrillar or necrotic basement membranes, accompanied by fine discrete electron-dense deposits, necrosis of inner (or intimal) SMC, hyperplasia and hypertrophy of outer SMC, and atrophy of a few scattered SMC. Infiltration with fibroblasts is a constant finding. McCormack and colleagues (1958) have found proliferation of

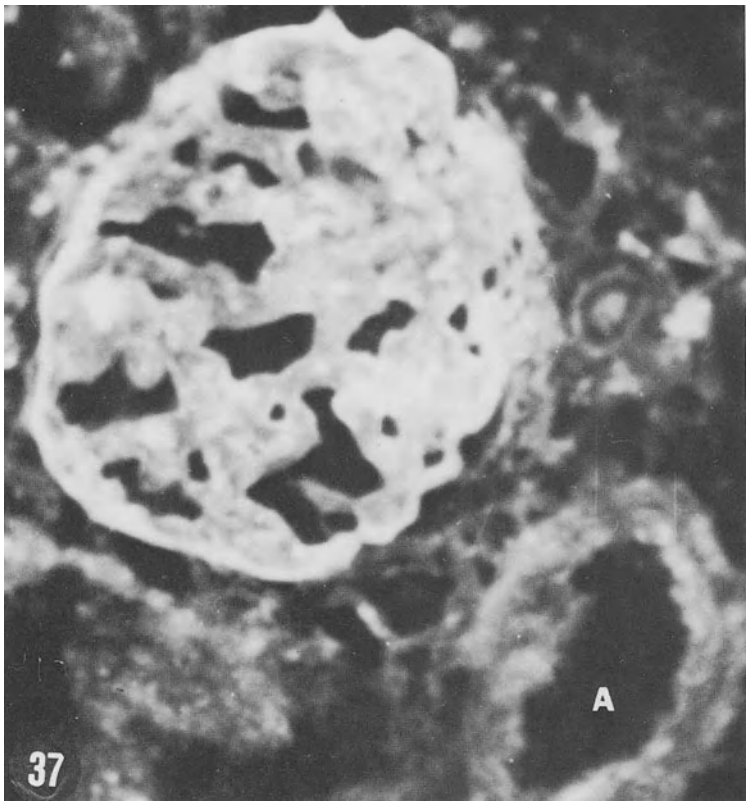

Fig. 9-37. In the glomerulus shown, 4+ fibrinogen is present diffusely and uniformly throughout the glomerular capillaries and the Bowman's membrane. The adjacent arteriole (A) shows slight or no fibrinogen (IFM, ×1600). From the same specimen as Fig. 9-36.

fibroblasts and excessive fibrous tissue in the intima (intimal fibroplasia), especially in small arteries, in a group of malignant hypertensive patients who received antipressor drugs for 4 to 48 months. Kojimahara and associates (1971) found similar changes when malignant hypertensive rats were treated with antipressor drugs. These authors have suggested that this type of change is associated with healing of acute lesions resulting from antihypertensive drug treatment. These vascular changes also were found in the patients studied in this series. Using EM and specific staining techniques, fibroblasts and excessive amounts of collagen fibers and elastic tissue were found in patients 5 through 10, who received vasodepressor drugs for variable periods.

We have stated that both proliferative and necrotic changes are found in the

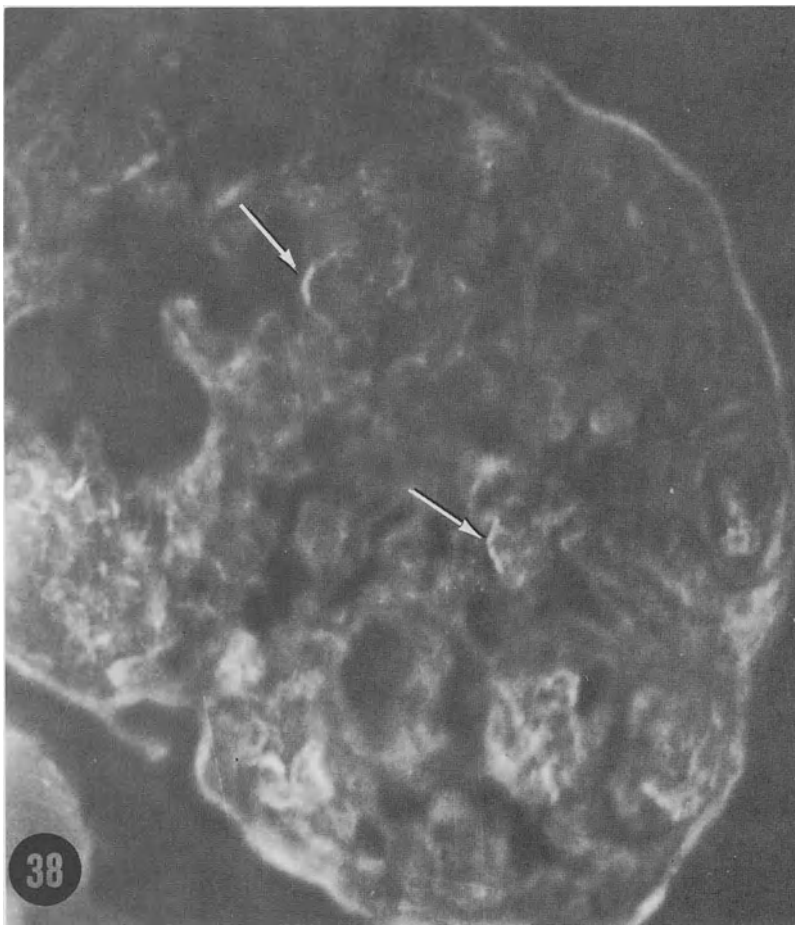

Fig. 9-38. After heparin treatment a few specks of fibrinogen (arrows) are seen in the glomerular capillaries (IFM, ×1600). From a 116-week-old SHR.

kidneys in malignant hypertension. Both changes can be identified in single arterioles if they are studied by EM.

Questions Pertaining to the Relationship between Hypertension and Renal Lesion

Does hypertension cause the renal lesions or do the renal lesions produce hypertension? Kimmelstiel and Wilson (1936) found a definite correlation between malignant hypertension and malignant nephrosclerosis. Heptinstall (1953) found vascular necrosis in patients with severe degrees of hypertension (diastolic pressure of 150 mm Hg or over). In contrast, Sommers and colleagues (1958) did not find a consistency between arteriolar necrosis and any specific level of diastolic pressure. Likewise, Kincaid-Smith *et al.* (1958) found artriolar necrosis in malignant hypertensive patients (by the criterion of bilateral papilledema) with systolic pressures less than 200 mm Hg. In attempting to answer this question, Wilson and Byrom (1939) studied contralateral kidneys from 35 rats from 5 days to 6 months after hypertension was produced by constricting one renal artery. These rats demonstrated necrotic changes in the arterioles resembling those observed

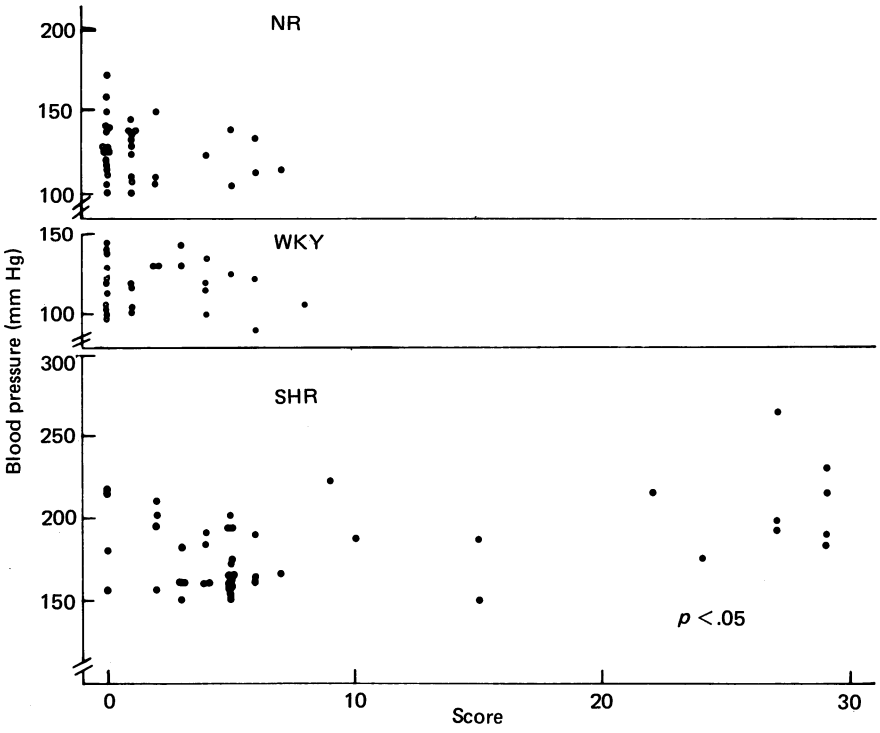

Fig. 9-39. Relationship between mean arterial blood pressure and histopathological scores in all rats: NR (normotensive Wistar rats), WKY (Wistar Kyoto rats), SHR (spontaneously hypertensive rats).

in human malignant nephrosclerosis. Recently Goldby and Beilin (1974) studied development and healing of arteriolar lesions using a tracer (carbon particles) and LM and EM techniques in rats made hypertensive by renal artery constriction and contralateral nephrectomy. Three types of lesions were observed: first, plasma and carbon particles had entered the media to displace and destroy SMC; second, additional intimal deposits containing plasma, fibrin, and macrophages appeared; third, the SMC were irregular in outline and surrounded by excessive extracellular material. Both groups of authors concluded from their observations that the necrotic arteriolar lesions were caused by severe hypertension.

The pitfalls of studies in experimentally induced hypertension have been discussed. Once again it should be stated that these experimental models are not comparable to essential hypertension. Until recently, no experimental animal model closely resembling essential hypertension was available. With the availability of the SHR (Okamoto and Aoki, 1963), an animal model closely resembling human essential hypertension, studies of the kidneys in SHR of all ages should provide a better understanding of the development of renal lesions and their relationships to the severity and duration of the hypertension. A significant number of young, severely hypertensive SHR showed normal renal histology or

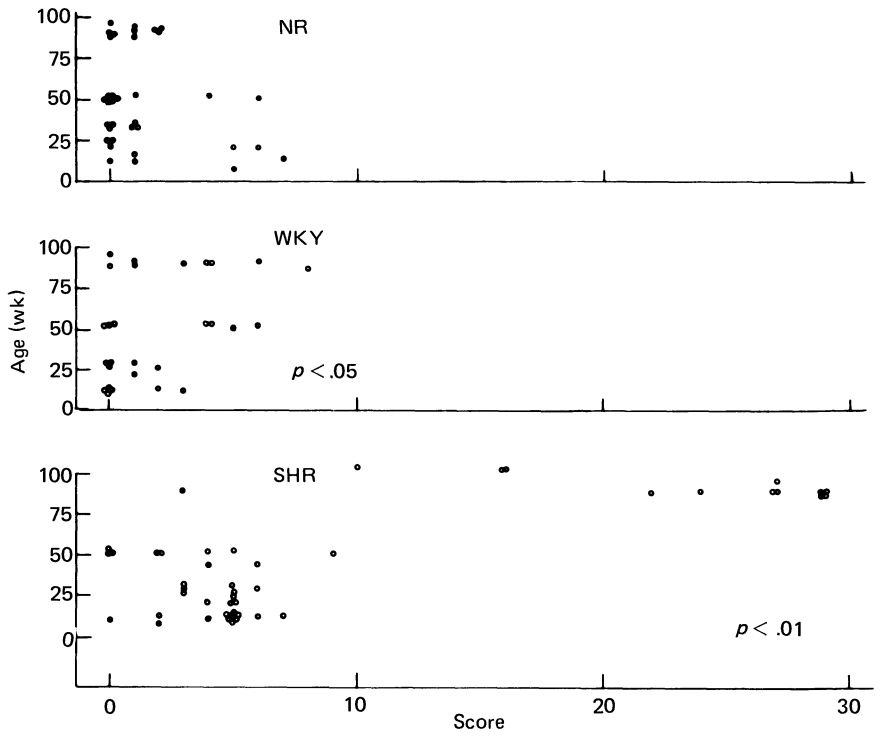

Fig. 9-40. Relationship between age and histopathological scores in all rats: NR (normotensive Wistar rats), WKY (Wistar Kyoto rats), SHR (spontaneously hypertensive rats).

mild renal lesions. Considering all ages, severe renal lesions consisting of polyarteritis, arteriolar necrosis, glomerular necrosis, and thrombosis appeared in the vast majority of old SHR, in contrast to no renal lesions in identically old NR or WKY.

The importance of severity of hypertension as a major factor in the pathogenesis of renal lesion can be rebutted by the demonstration of severe renal lesion in one patient with diastolic blood pressure of 110 mm Hg and mild renal lesion in another patient with diastolic blood pressure of 130 to 140 mm Hg. On the contrary, duration of hypertension can now be emphasized as an important factor which was not realized in the past by the evidence that progression of the renal lesions occurred over a 4-year period in one patient (#5) in this series. The highly significant association established between the severity of renal lesions and age (i.e., duration of hypertension) in SHR led to the conclusion that the development of severe (i.e., malignant) renal lesions in hypertension is more dependent on the duration of the hypertension than the severity of the hypertension. This conclusion is reinforced by the statement of Freis (1972), who suggested that age, heredity, and environmental influences, not hypertension alone, play roles in the severity of the renal lesions.

MECHANISMS OF RENAL LESIONS: HYPOTHESES

1. Renal lesions are secondary to hypertension. It is reasonable to conceive that genetic hypertension in the young animal (SHR) or in humans is not

Table 9-8
Previous Studies of Experimental Hypertension

Year	Authors	Species	Method of production of hypertension	Histology
1939	Wilson and Byrom	Rat	Unilateral renal artery constriction	Necrotic lesions (C[a])
1937–1939	Wilson and Pickering	Rabbit	Bilateral renal artery constriction	Necrotic lesions in extrarenal vessels
1941	Friedman *et al.*	Rat	Unilateral renal artery constriction	Necrotic lesions (C)
1942	Schroeder and Neumann	Rat	Unilateral renal artery constriction, and contralateral renal injury or disease	Proliferative or necrotic lesion (C)
1974	Goldby and Beilin	Rat	Renal artery constriction and contralateral nephrectomy	Intestinal, pancreatic vascular necrosis, deposits, infiltration, and edema (EM)

[a] Contralateral kidney.

associated with severe renal lesions because normal elastic tissue and SMC contents in the arterial vascular wall prevent development of severe lesions until the composition of the vascular elastic tissue and SMC components is altered by age. The presence of excessive amounts of elastic tissue, demonstrated by a specific staining technique in the renal arterioles of all SHR, with the most marked increases accompanied by disruptive changes in the vast majority of old SHR and malignant hypertensive patients (Figs. 9-41 and 9-42), lends support to this hypothesis. Sommers and associates (1958) stated that degeneration and disappearance of elastic tissue were accompanied by pooling of ground substance and its permeation into adjacent intercellular spaces. The causes for proliferation of SMC and overgrowth of collagen fibers and elastic tissue remain to be determined.

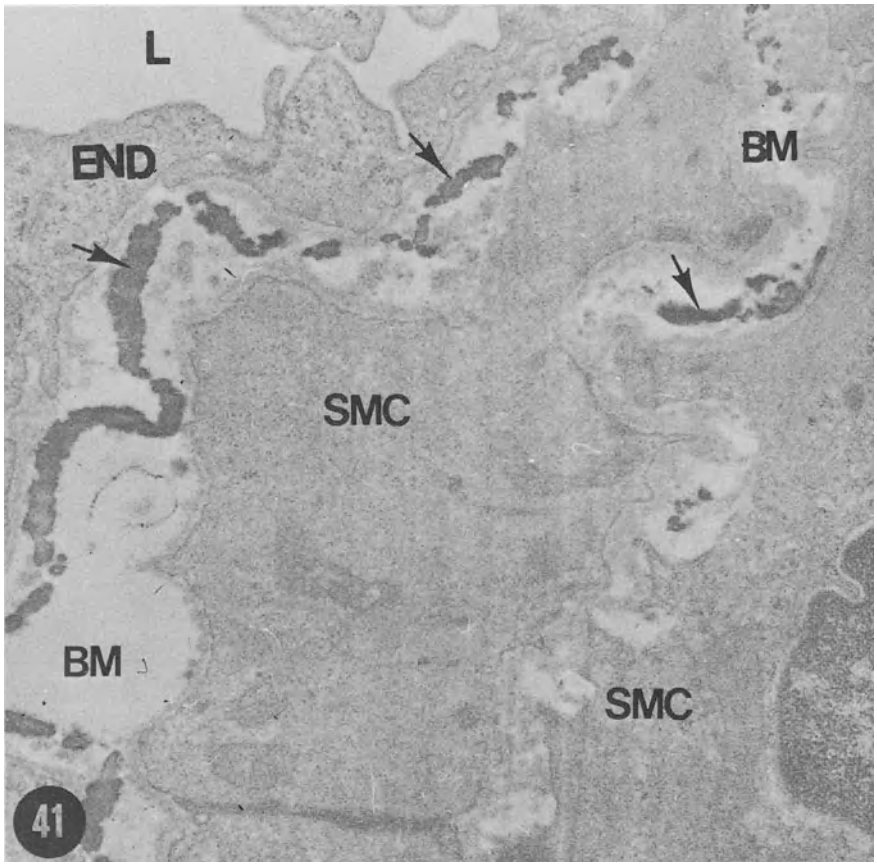

Fig. 9-41. This large arteriole from an old SHR kidney demonstrates excessive amounts of electron-dense elastic tissue (arrows) in the basement membrane (BM) between endothelial cells (END) and smooth muscle cells (SMC) and in the BM between individual SMC. The BM is swollen by a nonstainable substance, possibly edema (STPPS, ×18,000).

2. The mechanisms operative in the development of necrotic vascular lesions in the short period of weeks in renovascular hypertensive rats are unlikely to be the same as those causing necrotic renal lesions in SHR and hypertensive patients over a period of months or years. The possibility that angiotensin, often elevated in renovascular hypertensive humans and assumed to be elevated in these renal clip hypertensive rats, may produce ischemic (necrotic) renal lesions remains good. This hormone has been found to be normal in many malignant hypertensive patients and is consistently normal in SHR.

Almost all data reveal that renal lesions appear in the course of hypertension and are somehow caused by hypertension. In other words, renal lesions are secondary to hypertension. However, there is some evidence to suggest that renal lesions may be primary to hypertension, namely that hyperplasia and hy-

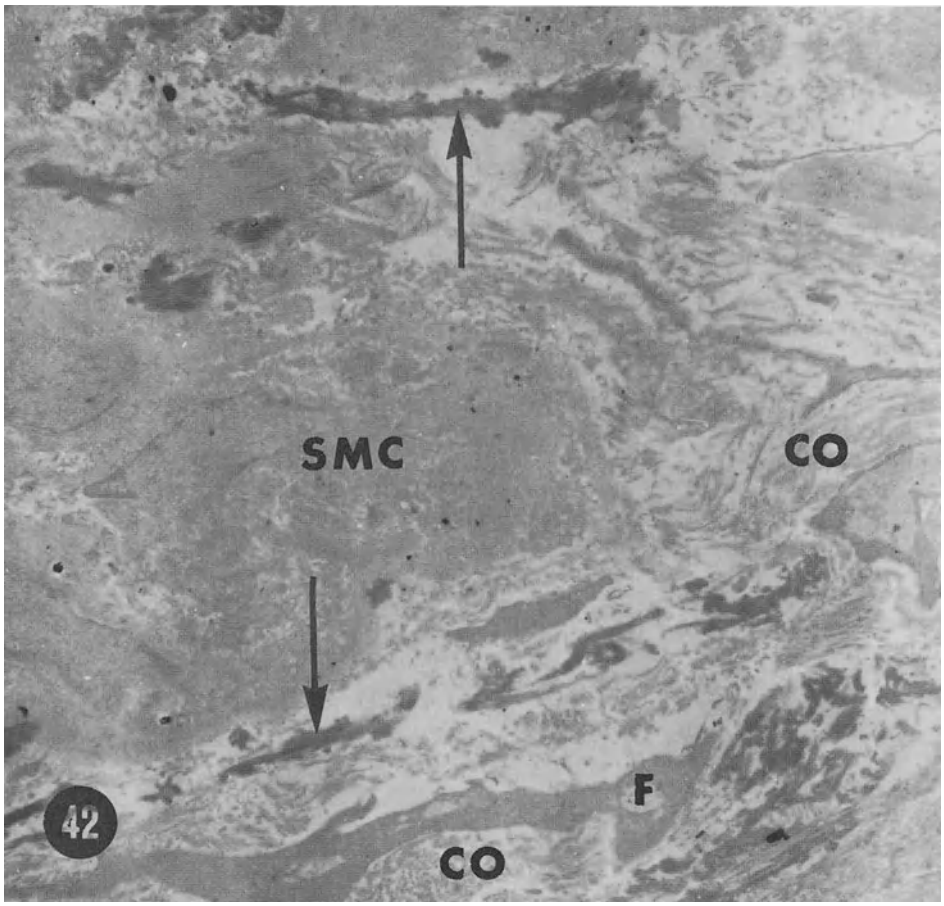

Fig. 9-42. In this renal arteriolar section from patient #8, excessive amounts of electron-dense elastic tissue (arrows) are seen surrounding the necrotic smooth muscle cells (SMC). Equally excessive amounts of non-electron-dense collagen fibers (CO), as well as fibroblasts (F) are also observed (STPPS, ×21,000).

pertrophy of SMC and excessive elastic tissue have been consistently observed in renal arterial vessels of SHR and malignant hypertensive patients. These changes may occur primarily in the arterial vessels, caused by some unknown stimuli, and may result in increased intramural tension with a consequent reduction of the intraluminal diameter of the arterioles. This anatomical alteration in the arterioles, especially the small arterioles, may explain increased peripheral resistance and higher diastolic pressure compatible with malignant hypertension. It is of some interest that excessive elastic tissue in the arterial vessel may be caused by decreased content or by inhibition of the enzyme elastase in severe hypertension. This proposal may be supported by the fact that intravenous administration of pancreatic elastase evoked a fall of blood pressure in the cat whereas the effects of larger doses were sometimes irreversible (Borsy *et al.*, 1959).

3. The association of fibrin deposition in the renal vessels and glomeruli with malignant hypertension is well known, but the causal relationship between this morphological abnormality and malignant hypertension is still debatable. The consistent presence of moderate to heavy amounts of fibrinogen (or fibrin)* in the glomeruli of all old SHR implies a relationship between glomerular thrombosis and hypertension. This theory is supported further by decreased glomerular thrombosis, accompanied by a fall in arterial blood pressures, in heparin-treated old SHR. Other investigators have reported similarities between renal lesions in malignant hypertension and those in normotensive scleroderma and thromobotic microangiopathies in humans. The latter two conditions often develop malignant hypertension, and this sequence has led to a hypothesis that thrombotic renal lesions precede malignant hypertension. The finding of glomerular thrombosis is a stimulating observation, since this is seen in conditions related to essential hypertension. There are also grounds to believe that glomerular thrombosis may indeed occur in hypertension. Preeclampsia and eclampsia which manifest essentially reversible hypertension are often accompanied by glomerular thrombosis Also, a decrease in fibinolytic activity has been found in preeclampsia. Fibrinolytic activity was not determined in SHR, but decreased fibrinolytic activity has been found in human essential hypertension (for details, see Mandal *et al.*, 1978*b*). Since SHR have great similarities to humans with essential hypertension, it seems logical to conceive that decreased fibrinolytic activity and glomerular thrombosis are determinant factors in the pathogenesis of essential (spontaneous) hypertension.

4. The positive fluorescence for IgG, γG, and C3 in the glomeruli in old SHR and in the glomeruli and arterial vessels of malignant hypertensive patients suggests an immunopathogenic mechanism of renal lesions in spontaneous and essential hypertension. Paronetto (1965) has suggested that deposition or formation of immune complexes is one of the mechanisms of tissue damage in malignant nephrosclerosis. In our study, glomerular IgG and γG were unchanged

* Fibrinogen is demonstrated by IFM, whereas fibrin can be detected by EM.

following heparin treatment in SHR, despite decreases in glomerular fibrinogen, MAP, and severity of arterial vascular lesions. Since renal lesions regressed following heparin treatment (although immunoglobulins remained unchanged), this would be more consistent with trapping of immunoglobulins rather than with a direct role in the pathogenesis of malignant hypertensive renal lesions.

RELATIONSHIP BETWEEN PATHOLOGY AND FUNCTIONAL FAILURE OF THE KIDNEY

Hypertensive patients with severe renal lesions frequently develop uremia. This contrasts with the normal or mildly elevated serum urea nitrogen and serum creatinine concentrations in SHR with vascular and glomerular lesions similar to those in malignant hypertensive patients. This raises a question of the mechanism of renal failure in hypertension. Here again, there is no uniform agreement on the impact of the lesions of individual components of the kidney as determinants of functional failure. Kimmelstiel and Wilson (1936) suggested that terminal renal failure was related to the arteriolar necrosis more closely than to the hypertension. McCormack *et al.* (1958) have stated that intimal cellular proliferation and intimal fibroplasia with marked narrowing of the lumina in small arteries and large arterioles both lead to slowly progressive renal failure. Kincaid-Smith (1975) found good correlation between the proliferative arteriolar lesion and renal failure. In fact, the proliferative arteriolar lesion is a more prominent feature in hypertensive patients with renal failure than in those without renal failure. Also, this type of arteriolar lesion is a less striking feature in SHR and the rats do not develop renal failure. Even though the proliferative arteriolar lesion with intimal fibroplasia is a consistent finding in the kidneys of hypertensive patients with renal failure, it is difficult to attribute the renal failure to arteriolar lesions alone since the glomeruli are also severely damaged. Furthermore, the influence of widespread loss of tubules and interstitial changes found in severe hypertensive patients must be remembered in considering pathogenesis of renal failure. SHR with severe arterial vascular and glomerular lesions had only slight tubulointerstitial changes and revealed no more than mild azotemia. In SHR, serum urea nitrogen concentrations ranged from 19 to 41 mg/100 ml, with a mean value of 27 mg/100 ml (normal 20–22 mg/100 ml). Wilson and Byrom (1939) also did not find discernible damage in the tubules and interstitium of rats with experimentally induced hypertension, and the blood urea values in hypertensive rats were not different from those in control rats.

The diffuse and widespread tubulointerstitial lesions in patients 6 to 10 provided a picture greatly different from that observed in the SHR, where preservation of most tubules and only mild interstitial changes were found. These two studies suggest some relationship between preservation of tubular mass and interstitium and maintenance of normal renal function. In other words, the renal functional impairment seen in severe or malignant hypertensive patients can be

associated with a decreased number of tubules, damage in the remaining tubules, and moderate to marked infiltration of the interstitium by fibrous tissue and mononuclear cells. A similar disparity between the morphological findings and the degree of nitrogen retention has been described by Risdon and colleagues (1968). These authors found the best correlation between loss of tubules and changes in the interstitium, and in plasma creatinine concentration or creatinine clearance. If this is true, then rapid deterioration of renal function observed in some of the patients with severe or malignant hypertension may be explained on the basis of superimposed acute tubular necrosis. This may account for the dramatic deterioration of renal function in patient #5, who maintained stable renal function for a long time. Acute tubular necrosis has also been observed by other investigators in patients with accelerated hypertension who developed acute renal failure (Sevitt *et al.*, 1971).

SUMMARY

1. This communication presents data on light microscopy (LM) and electron microscopy (EM) studies of kidneys from essential benign (BH) and malignant (MH) hypertensive patients and spontaneously hypertensive rats (SHR).

2. In BH, the hyaline thickening (PAS-positive matrix) of arterial vessels observed by LM is found to consist of thickened basement membrane (BM), excessive BM-like material, and atrophic smooth muscle cells (SMC) when the vessel is examined by EM. These arteriolar changes appear nonspecific and unrelated to hypertension because similar changes can be found in the ischemic kidney and in chronic renal disease without hypertension.

3. An array of proliferative and necrotic arterial vascular lesions, along with obsolescence and necrosis of glomeruli, marked tubular atrophy, and interstitial changes, are found most commonly in clinical MH (with papilledema), but such renal lesions are not uncommon in clinical BH (without papilledema). Thus, clinical malignant hypertension and necrotic or proliferative renal arteriolar lesions are often related, but not in a clear cause and effect association. In our own study, both hyperplasia and necrosis of renal arteriolar SMC have been found in clinical malignant hypertension. Both types of arteriolar changes may be found to coexist in the same arteriole, especially when the vessel is examined by EM.

4. The cause and effect relationships between hypertension and the associated renal lesions remain undetermined. Our study and those of other authors have failed to demonstrate any clear correlation between severity of renal lesions and severity of the hypertension. However, in the SHR study, we found that younger rats had milder lesions. Considering all ages, severe renal lesions resembling those in clinical malignant hypertension appeared in old SHR, and a highly significant association was established between age (i.e, duration of hypertension) and pathological changes. A tentative relationship between the renal lesions and

the duration of hypertension has been demonstrated in this and another study (Sinclair *et al.*, 1976) in humans. Thus, it appears that renal lesions in hypertension are more dependent on the duration of the hypertension than on the severity of the hypertension.

5. Azotemia is a consistent finding in hypertensive patients with severe renal lesions. The arterioglomerular changes are similar in clinical malignant hypertension and in old SHR; the absence of tubulointerstitial changes and azotemia in SHR contrasts strikingly with the massive tubular loss, interstitial infiltration and fibrosis, and progressive azotemia observed in hypertensive patients. This disparity suggests that renal failure occurs more from loss of tubules and interstitial changes than from arterioglomerular lesions.

6. Fibrin and platelet aggregates (thrombosis) are inconspicuous in the arterioles, but they are found abundantly in the glomeruli associated with severe renal lesions in hypertensive humans and SHR, especially the latter. The role of glomerular thrombosis in the development or acceleration of the renal lesions or hypertension remains to be determined.

7. "Fibrinoid necrosis" appears to be an inappropriate term because this LM appearance resolves, by EM, as necrotic SMC with slight or no fibrin and with or without electron-dense deposits.

8. EM study can characterize the early stage of severe arteriolar lesions by demonstration of collagen fibers, excessive elastic tissue, and hyperplasia of SMC when LM study reveals intimal thickening or mild SMC hyperplasia. Therefore, the specific renal lesions of hypertension can be distinguished from the nonspecific renal changes associated with benign hypertension by EM study, even at an early stage.

9. The study of renal pathology in the SHR, which is considered the best animal model yet developed for the study of clinical essential hypertension, has provided further evidence underscoring the pertinence of this model for understanding renal pathology in essential hypertension.

PART 2
A PERSPECTIVE OF THE ANTIHYPERTENSIVE FUNCTION OF RENAL MEDULLARY AND PAPILLARY INTERSTITIAL CELL AND GRANULE

BACKGROUND

The observation that arterial blood pressure rises to hypertensive levels in dogs following bilateral nephrectomy (renoprival hypertension) has warranted investigation concerning the protective function of the normal kidney against hypertension. This apparent function of the normal kidney was recognized when

explantation of the normal whole kidney, whole renal medulla, or outer medulla was found to minimize or even prevent renoprival hypertension in dogs. These experiments suggested that the normal whole kidney or normal renal medulla might liberate vasodepressor factor(s) which mitigate against hypertension. A fall in blood pressures was noted following intravenous administration of renomedullary extract into renoprival hypertensive dogs and transplantation of live histocompatible renal medulla into experimentally induced hypertensive rats. Upon removal of the medullary graft, blood pressures returned to hypertensive levels. These observations have led Muirhead and associates (1970) to state that the vasodepressor or antihypertensive factors are located in renal medulla.

This hypothesis has stimulated morphologists to search the site of production of the vasodepressor substance(s) in the renal medulla. Conspicuous granules inside numerous interstitial cells (IC) located in the inner medullae and papillae were proposed by Muehrcke and associates (1970) as the source of the suspected vasodepressor substance(s). Histologically, these granules revealed lipoid characteristic after intense staining with Sudan black B, oil red O, and osmium. The vasodepressor substances isolated from the renal medullary extract have been found to contain mainly lipid. Therefore, the abundant lipoid granules present inside the IC of renal medulla appear to be the most probable source of the medullary vasodepressor substance(s).

ANATOMY OF THE INTERSTITIAL CELLS AND GRANULES

The rat has a single conspicuous papilla in each kidney. In dog and human subjects, each kidney has half a dozen papillae but they are not as noticeable as in rats. The papilla is rich in interstitial cells, and stellate processes (perhaps detached segments of IC). The IC are located between loops of Henle or a loop of Henle and a peritubular capillary, or between two collecting tubules. They are found in highest numbers at the papillary tip, especially in rats. The IC vary in size and shape, and are characterized by long cytoplasmic processes, single nuclei occupying one-third to one-half of the cell, many prominent rough-surfaced endoplasmic reticula, Golgi complexes, mitochondria, and, of course, the most striking feature, a large number of granules (Figs. 9-43 to 9-45). The number of granules per cell varies from 2 to 40. The IC and granules are always more numerous in rat kidneys than in human kidneys. In human kidneys no more than three to four granules per cell have been observed (Fig. 9-46). The IC and granules are even fewer in dog kidneys (Fig. 9-47). A variable number of extracellular granules (free granules) have been observed in rat and human kidneys. These free granules are located adjacent to interstitial cell or stellate process and are not surrounded by either cytoplasmic constituents or cell membranes (Fig. 9-44). Also, they are found to be located along the tubular and capillary basement membranes, as well as inside the tubular and capillary lumina. The free granules are identical to intracellular granules and appear to have been extruded from IC.

Three types of granules—layered, homogeneously dark, and gray—have been recognized inside the IC (Figs. 9-43 to 9-47) and all but gray have been recognized in the free interstitium. It is well known that the binding of osmium to lipid (especially unsaturated fatty acids) renders the lipoid structure electron dense. Since the granules are lipoid in nature, it appears that the variable appearance of the granules, i.e., layered, homogeneously dark, and homogeneously gray, is most likely to be due to a difference in the amounts of saturated and unsaturated fatty acids in the three types of granules.

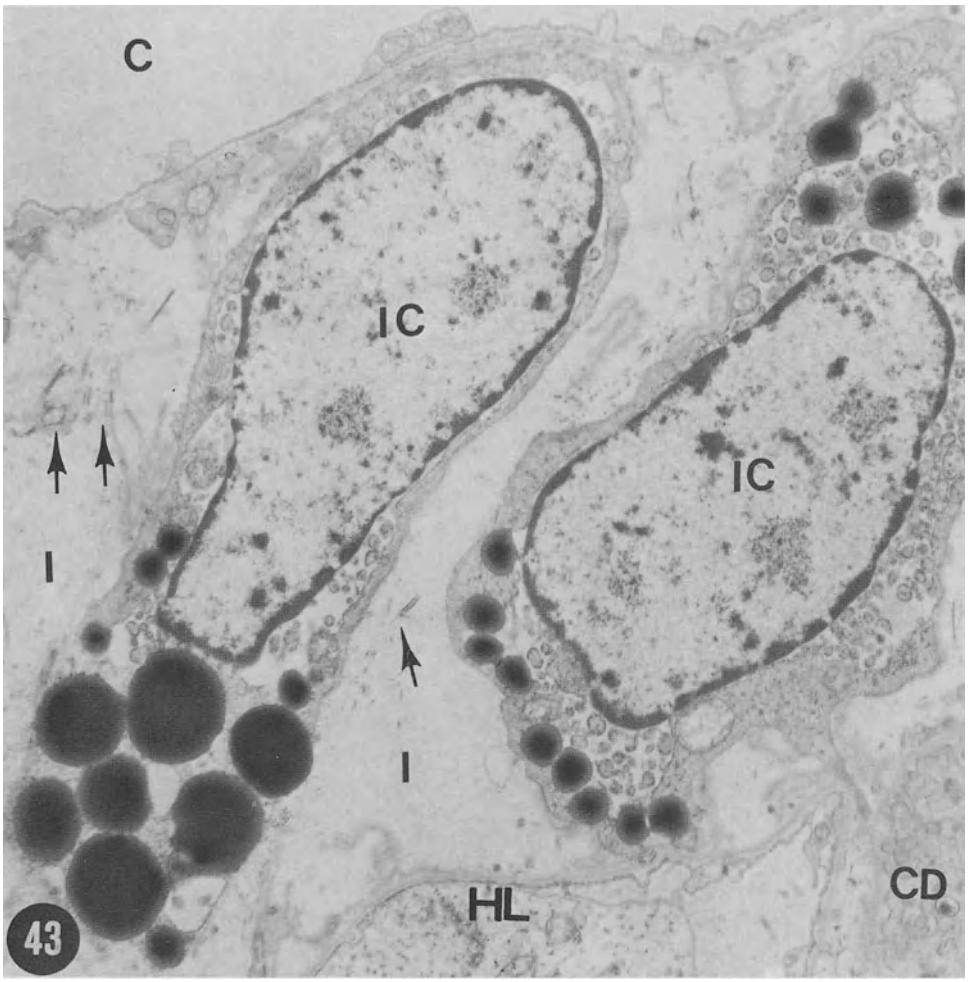

Fig. 9-43. Renal papilla from a normotensive Wistar rat (NR). Two adjacent interstitial cells (IC) are located between a peritubular capillary (C) on one side and Henle's loop (HL) and collecting duct (CD) on the other side. Both the IC contain numerous triple-layered granules. A few collagen fibers (arrows) are seen in the interstitium (I) (UA + LC, ×16,000).

There is some evidence to indicate that the IC play an important role in concentration and dilution of urine. They appear to be secretory because of the presence of rough-surfaced endoplasmic reticulum, Golgi complexes, and characteristic granules. Muirhead and associates (1975) believe that the active principle, which is extractable from fresh renal medulla and designated as the antihypertensive neutral medullary lipid, appears to be an attractive potential

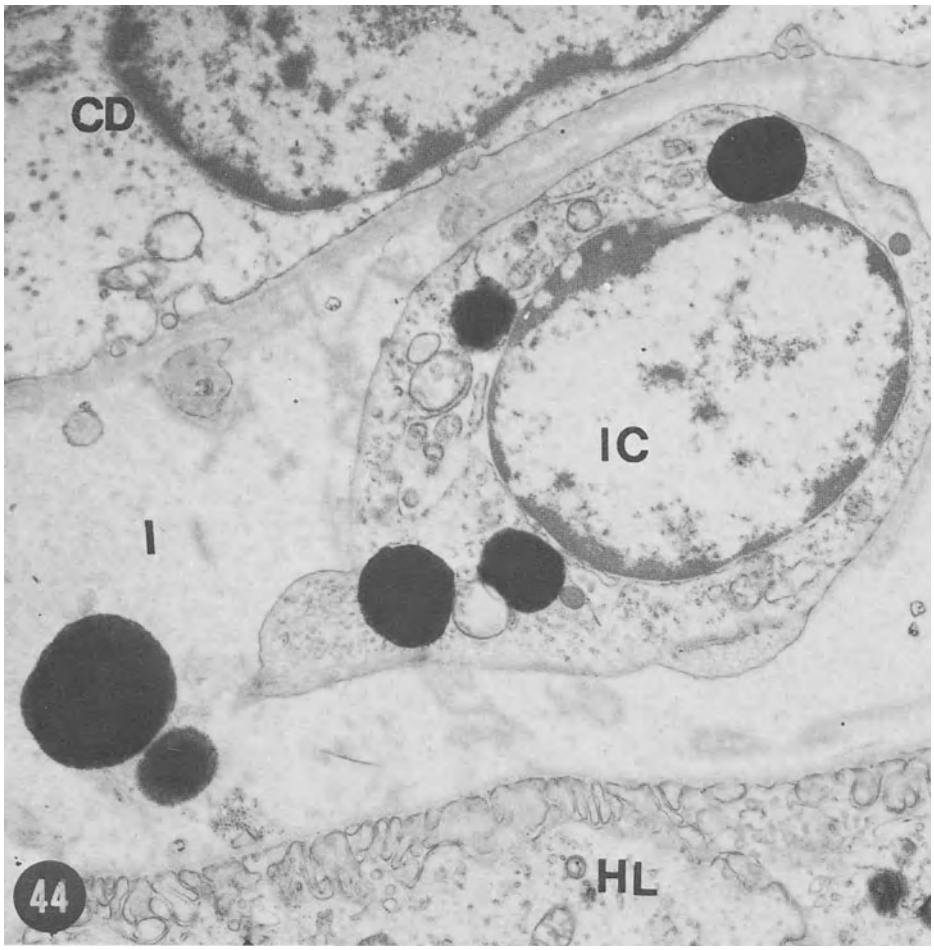

Fig. 9-44. An interstitial cell (IC) from a young SHR kidney is located between Henle's loop (HL) and collecting duct (CD); it has a few homogeneously dark granules. The free (extracellular) granules in the interstitium (I) are identical in appearance to the intracellular granules (UA + LC, ×18,000).

mediator of the antihypertensive function of medullary and papillary IC. This hypothesis had been tested in part by quantitative analyses of the interstitial cells and their granules in hypertensive states.

RELATIONSHIP OF RENAL MEDULLARY AND PAPILLARY INTERSTITIAL CELLS TO HYPERTENSION

Studies in Experimentally Induced Hypertensive Rats

Hypertension was induced in rats by feeding them excessive amounts of sodium chloride or by intramuscular administration of deoxycorticosterone acetate (DOCA) or both, or by unilateral renal artery constriction. Systolic pressures were serially measured, and after weeks or months of sustained hypertension,

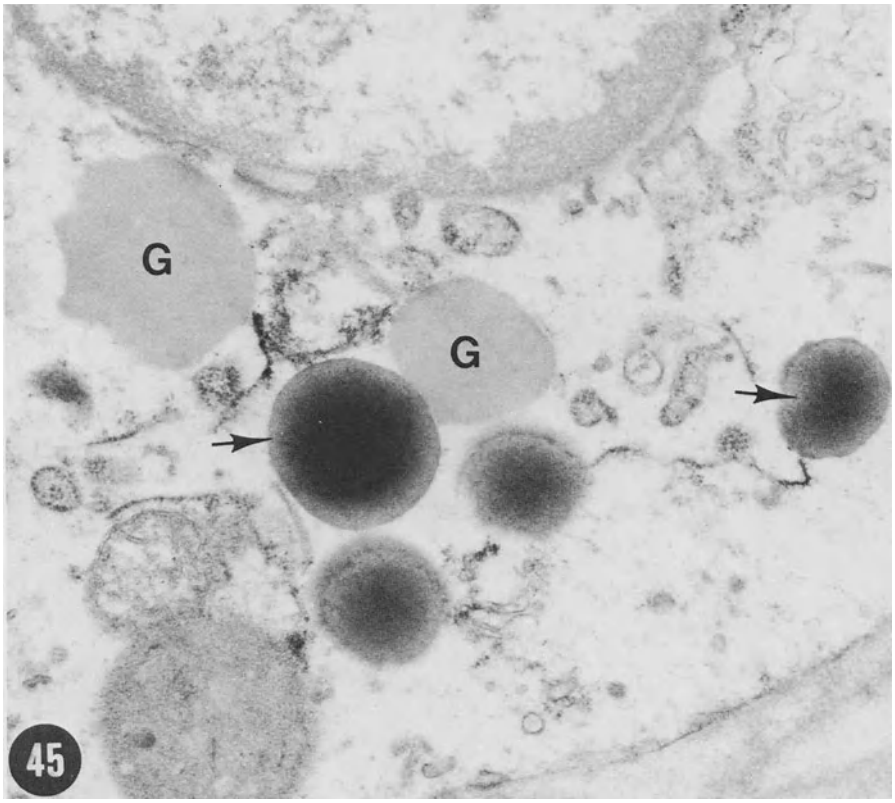

Fig. 9-45. An interstitial cell from a young SHR kidney demonstrates a combination of layered and gray granules. The layered granules (arrows) are composed of a central electron-dense area surrounded by a peripheral zone of lighter electron-dense material; the gray granules (G) consist of a uniform lighter electron-dense material (UA + LC, ×22,000).

the animals were sacrificed. The kidneys were removed and fixed for LM and EM studies. The granules of the interstitial cells were quantified and the granular counts in the hypertensive animals were compared with those in the normotensive control animals. All the studies consistently demonstrated significant reduction of interstitial cellular granularity in hypertensive rats and suggested that decrease in granularity was secondary to hypertension.

Studies in Spontaneously Hypertensive Rats

Even though the reduction of interstitial cellular granularity in experimentally induced hypertensive rats is considered a secondary change, the cause and effect relationships between the reduced interstitial cell granules and hypertension have

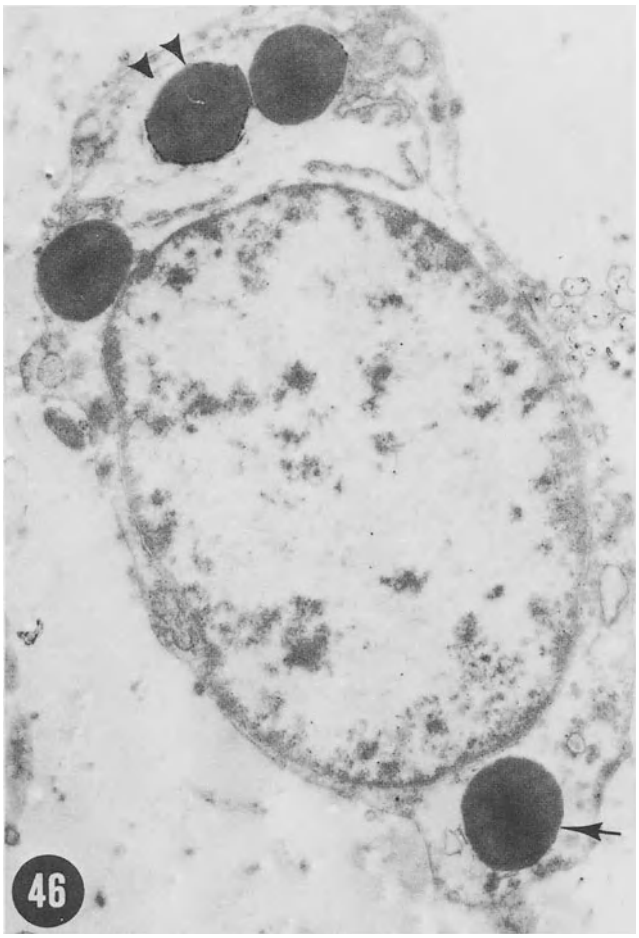

Fig. 9-46. Papillary interstitial cell from a normal human kidney shows layered granules (arrow) and gray granules (arrowheads) (UA + LC, ×16,000).

remained unclear. We extended our investigation into the spontaneously hypertensive rat, studying the interstitial cells of the renal medulla and papilla from SHR 10 to 33 weeks of age and from age- and sex-matched normotensive NR, using electron microscopy and utilizing a variety of staining techniques. SHR kidneys revealed significant reduction of interstitial cell granularity (Table 9-9). Further studies of the whole renal papilla from SHR and age-matched NR demonstrated a reduction of the total number of interstitial cells and granularity in SHR. The significantly greater number of gray granules than layered and homogeneously dark granular types in SHR and the presence of many free granules in SHR appear to be interesting. The gray granules, being devoid of membrane and being irregular in shape, may represent the "chemical end product" of the other two

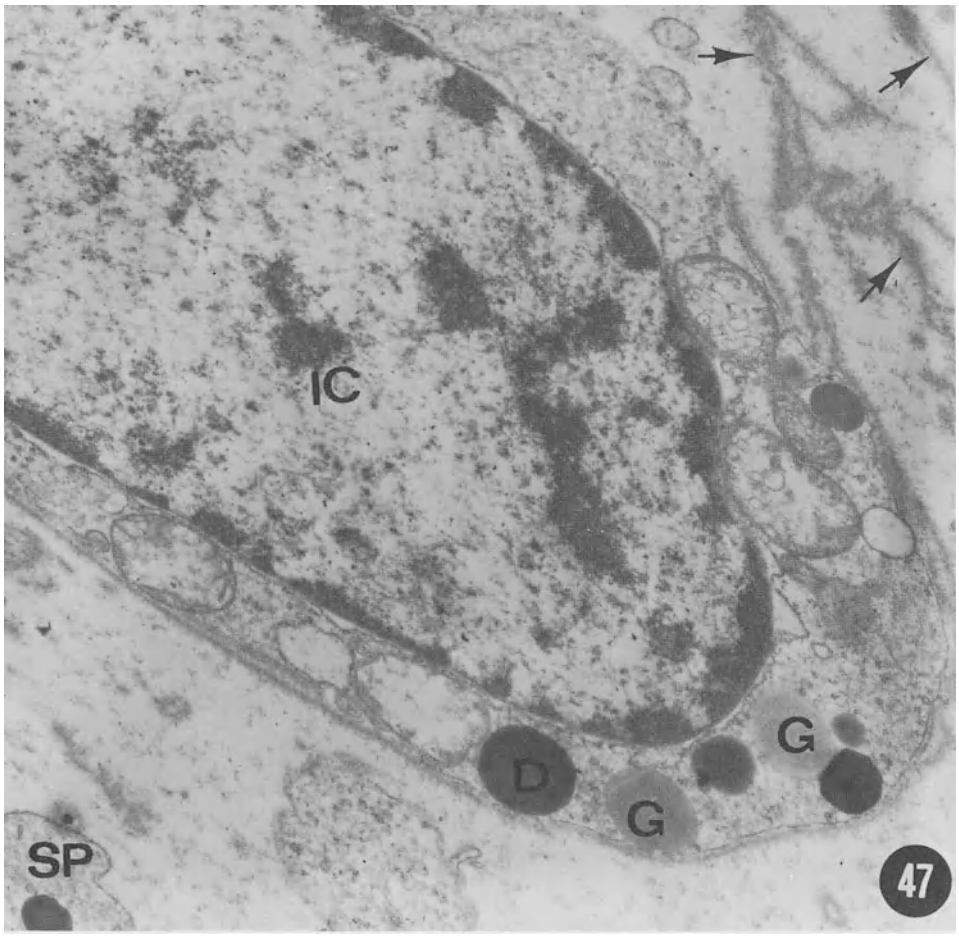

Fig. 9-47. This interstitial cell (IC) from dog renal papilla reveals homogeneously dark granules (D) and gray granules (G) within the IC and dark granules within a stellate process (SP). Collagen fibers (arrows) are shown (UA + LC, ×22,000).

granular types. Therefore, the presence of a greater number of gray granules in the papilla of SHR suggests a decrease of lipid material in SHR kidneys. Since the papillary granules are suspected to be the source of vasodepressor substance(s), a decrease of the lipid content of the granules may be commensurate with reduction of the renal medullary vasodepressor substance(s). Furthermore, the presence of many layered and homogeneously dark granules in the urine of SHR, compared to almost complete absence in NR urine, suggests a greater loss of the proposed vasodepressor material in SHR. These findings are in agreement with an observation made by Tobian and Azar (1971). They found, in their experiment, that papillae of "post-salt" hypertensive rats released more prostaglandins than the papillae of normal control rats. The authors have suggested that the hypertensive rats tend to secrete more vasodepressor substance(s) in an attempt to alleviate hypertension.

MECHANISM(S) OF THE REDUCTION OF INTERSTITIAL CELLS AND GRANULES IN THE KIDNEYS OF HYPERTENSIVE RATS

Since the IC granules have been found to be reduced in both experimentally induced hypertensive rats and spontaneously hypertensive rats, it is assumed that the granular reduction is secondary to hypertension. At a glance, it seems right, but it becomes a difficult task to explain how hypertension reduces the papillary interstitial cellular granularity. Papillary ischemia resulting from hypertension was thought to be the cause of changes in the papillary interstitium. If papillary ischemia is suspected on the basis of cortical lesions which may be a direct effect of hypertension, then it would be difficult to compare the reduced granularity in experimentally induced hypertensive rats with that in SHR; for example, young SHR demonstrated significant reduction of papillary interstitial granularity, although their renal lesions were mild. This contrasted with severe renal lesions in experimentally induced hypertensive rats. Because of a disparity in the severity of renal lesions between experimentally induced hypertensive rats

Table 9-9
Analysis of Renal Papillary Interstitial Cell Granularity

Index	Normal Wistar rat (mean ± SEM)	Spontaneously hypertensive rat (mean ± SEM)
Average number of granules per cell	7.1 ± 0.49	4.6 ± 0.68[a]
Percentage of cells with greater than 10 granules per cell	16.7 ± 4.7	6.2 ± 2.4[a]
Percentage of agranular cells	7.9 ± 3.6	30.0 ± 7.2[a]

[a] $p < 0.05$.
Source: From Mandal *et al.* (1974).

and SHR, reduction in papillary interstitial granules cannot be explained on the basis of papillary ischemia alone. How can papillary granular analysis in a rat with mild or no renal lesion be compared with the result in another rat whose kidneys demonstrate severe hypertensive renal lesions? Therefore, the reduction of papillary interstitial granularity seems to be caused by a different mechanism in SHR and other experimental hypertensions.

SIGNIFICANCE OF THE STUDIES OF RENAL PAPILLARY INTERSTITIAL CELLS IN RATS

Application to Human Subjects

We were able to study papillary interstitial cells and granules in kidneys removed from patients with malignant hypertension, or tumor of the kidney or urinary conduit. In hypertensive kidneys the cells were fewer and agranular. Even so, it became a difficult task to distinguish IC from a variety of infiltrating cells such as fibroblasts. Tumorous kidney was less than optimum for control study. Therefore, studies of interstitial cells and granules from normal human kidneys were deemed necessary for the purpose of comparison of IC and granule data between SHR and essential hypertensive patients.

Obstacles to Human Study

1. Percutaneous renal biopsy is seldom successful in securing papillary tissue. It is not rare to find medullary tissue in the percutaneous biopsies, but hardly, if ever, is the medullary tissue studied by EM when the purpose of the biopsy is study of the glomeruli.

2. The failure to acquire good information on renal papillary interstitial cells in humans is largely caused by the suboptimum condition of the autopsy materials for EM study. Even light microscopy study of the autopsy materials is unsuitable for this purpose, since certain cell organelles, e.g., mitochondria, undergo rapid autolysis. Autolytic tissue produces vacuoles, which may be misinterpreted as granules by light microscopy.

Recently, we were able to study papillary interstitial cell and granule from the kidneys of four young healthy subjects who died in accidents. Admission physical examinations and laboratory tests were normal. All of them had good urinary output. Kidneys were removed soon after cerebral death when the heart was beating. LM study of the routine histological sections of the kidneys was normal in all subjects. The papillary interstitial cells and granules are not so numerous as in rats. As in rats, three types of granules have been observed; however, layered and gray granules are uncommon (Fig. 9-46).

Prospects for Definition of the Relationship between Interstitial Cell or Granule and Hypertension

1. Studies of IC and granules in SHR of all ages (4–100+ weeks).
2. Studies similar to the foregoing in SHR, untreated and after treatment for a prolonged period with vasodepressor drugs.
3. Serial biopsy studies in hypertensive patients. (This is most difficult to achieve for the reason stated earlier.)

Since the IC and granules of human kidneys resemble those in normal rats and SHR, it seems justified to rely on the data derived from rat studies.

CRITICAL QUESTIONS RELATIVE TO RENAL MEDULLARY VASODEPRESSOR SUBSTANCE AND ITS SOURCE

There is enough evidence to indicate the presence of vasodepressor substance(s) in the renal medulla of dogs and rats. The renal cortex in these animals appears to be devoid of this function. The vasodepressor substance is lipid in nature and appears to be consistent with prostaglandins or with one or more precursors of prostaglandins. Young SHR tend to have higher prostaglandins than young WKY controls. It has been proposed that eventual deficiency of prostaglandins occurs because of overutilization in an attempt to compensate for hypertension in SHR. This idea, however, has not been confirmed. Since electron microscopy and histochemistry studies demonstrate lipoid characteristics of papillary interstitial granules, it seems logical to hold these granules as the potential source of prostaglandins or precursors. Further support is offered by the consistent finding of the reduction of papillary granularity in hypertensive rats. Because the interstitial cell is enriched with granules and has other secretory components, e.g., rough-surfaced endoplasmic reticulum and mitochondria, to name some, the IC has been advocated as the secretory source of the renal vasodepressor substance or prostaglandins.

The following evidence indicates that the human kidney may possess this vasodepressor function: (1) the high concentration of prostaglandins in human renal papillae, (2) the similarity of the IC and granules in human papillae to those in rat renal papillae, and (3) the normalization of blood pressure following transplantation of renal allografts in many hypertensive patients.

There are some observations which apparently contradict the idea of the renal vasodepressor function in human subjects. The renal medullary nodule from hypertensive patients was found to consist of fibroblastic-type cells. However, there was no difference in blood pressure and heart weight in the patients with renal medullary nodule and those without it. There are some pitfalls in this study, such as the lack of EM observation, and the fact that fibroma as it appears may not be composed of IC; these must be considered seriously before emphasizing the value of this study.

SUMMARY

1. It is known that renoprival hypertension occurs in dogs; it is unknown whether or not it occurs in humans.

2. Renoprival hypertension was prevented or minimized following explantation of a whole kidney or renal medulla in dogs; fall in blood pressure was noted following intravenous administration of renomedullary extract into renoprival hypertensive dogs and transplantation of live histocompatible renal medulla into experimentally induced hypertensive rats; prompt fall of blood pressure has been noted after transplantation of allografts into hypertensive patients.

3. Evidence is overwhelming in favor of the presence of vasodepressor substance(s) in the normal renal medulla of animals, *but is not strong in humans*. The vasodepressor substance(s) appears to be prostaglandin or its precursors.

4. The renal medullary and papillary interstitial cells have been implicated as the source of proposed vasodepressor substance(s).

5. The papillary interstitial cells and granules in normal human kidneys resemble those in spontaneously hypertensive rats (SHR); SHR is considered as the ideal model for human essential hypertension and has shown reduction of granularity at a young age.

6. Owing to the similarities between the spontaneously hypertensive rat and essential hypertensive humans, serial studies of papillary granularity in SHR of all ages should be done to demonstrate explicitly its role, if any, in the pathogenesis of spontaneous (essential) hypertension.

ACKNOWLEDGMENTS. Figures 9-14 to 9-17 are reproduced from Mandal *et al.* (1977*b*), by permission of the editor of *Annals of Clinical Laboratory Science*. Figures 9-2, 9-5, 9-6, 9-10, 9-12, 9-18, 9-24, 9-28, and 9-32 are reproduced from Mandal *et al.* (1977*c*), by permission of the editor. Figures 9-31, 9-33, 9-37, and 9-38 are reproduced from Mandal *et al.* (1978*b*), by permission of the editor of *Microvascular Research*. Figures 9-44 and 9-45 are reproduced from Mandal *et al.* (1974, 1975), respectively. Figures 9-39 and 9-40 are reproduced from Mandal *et al.* (1977*a*).

The specimen depicted in Figs. 9-17 to 9-22 was kindly provided by Robert C. Muehrcke, M.D., West Suburban Hospital, Oak Park, Illinois.

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Chronic Pyelonephritis 10

INTRODUCTION

Chronic pyelonephritis is one of the most perplexing subjects encountered in the practice of nephrology. The confusion is caused by ambiguous ideas concerning pathogenesis, a lack of absolute histopathological features, and sampling errors in the biopsy specimens used to diagnose the condition in the living individual. In general, chronic pyelonephritis may be defined as a pathological state of one or both kidneys as a result of infection by bacteria or other microorganisms, or injuries caused by noninfectious agents such as chemicals. There is much debate over whether chronic pyelonephritis is a separate entity or a variant of chronic interstitial nephritis. This is not a result of the similarities in histological appearances so much as the failure to document bacterial infection as the cause of chronic pyelonephritis in the vast majority of cases. Bacterial infection is always suspected but seldom has it been confirmed in the renal tissue. Many investigators have begun to believe that factors other than infection alone cause chronic pyelonephritis. Therefore, it has been proposed and accepted by many individuals that chronic interstitial nephritis rather than chronic pyelonephritis is a more appropriate term. Since in many cases no identifiable cause is found to account for the renal histopathology, it seems logical to substitute the term idiopathic chronic pyelonephritis for bacterial chronic pyelonephritis.

This chapter is concerned with three important facets: (1) etiology, (2) pathology and differentiation between chronic pyelonephritis and chronic interstitial nephritis, and (3) perspectives of chronic pyelonephritis in the clinical practice of nephrology.

ETIOLOGY

There is an increasing tendency to believe that chronic pyelonephritis is one of the types of chronic interstitial nephritis. This is mainly a result of the similarities of the histological features of chronic pyelonephritis and other types of chronic interstitial nephritis. In an analysis of the etiological factor in 101 patients with chronic interstitial nephritis as reported by Murray and Goldberg (1975) (see Table 10-1), chronic interstitial nephritis was caused by infection in only 27% of the patients. The evidence for this was the history of two documented urinary tract infections sought in each of these patients. In half of them anatomical abnormalities were found; in one-quarter of them, stone was found in the urinary tract. Therefore, in three-fourths of the patients, urinary tract infection was secondary to an obstructive state of the urinary conduit. While 32 of 34 patients with anatomical abnormalities who had chronic interstitial nephritis showed varying degrees of obstruction, only two demonstrated ureteral reflux without demonstrable obstruction. There is every reason to suspect that in all these patients obstruction (due to anatomical abnormality) coupled with infection led to chronic interstitial nephritis. These two groups are similar, and if they are lumped together, it appears that 53% of the patients with chronic interstitial nephritis had obstructive features such as anatomical abnormality or stone.

Traditionally chronic pyelonephritis can be divided into (1) obstructive form and (2) nonobstructive form. The obstructive form is common in adults, especially the elderly, but is quite uncommon in childhood. Common causes of urinary tract obstruction include prostatic hypertrophy; stricture of urethra; bladder outlet obstruction by stone; tumor; or ureteral obstruction by stone, constriction due to traumatic scar, tumor, or idiopathic fibrosis. Less common causes are intramural ureteral tumor, congenital anatomical abnormalities such as urethral valve, ure-

Table 10-1
Etiologic Factors of Interstitial Nephritis in 101 Patients

Factor	Primary cause[a] (no.)	Secondary factor[b] (no.)
Anatomic abnormalities	31	0
Analgesic abuse	20	0
Hyperuricemia	11	0
Nephrosclerosis	10	7
Stone	9	3
Sickle cell disease	1	1
Renal tuberculosis	1	0
Bacterial urinary tract infection	0	27
Multiple	7	
Idiopathic or indeterminate	11	

[a] Only or initial etiologic factor present in indicated number of patients.
[b] Occurred subsequent to primary cause.
Source: From Murray and Goldberg (1975), by the kind permission of Dr. M. Goldberg.

teral diverticula, and aberrant renal artery with compression of the ureter. Chronic retention of urine and repeated catheterization comprise the major cause of chronic pyelonephritis and chronic renal failure among the patients with paraplegia and autonomic neuropathy. Although it is true that repeated catheterization is the cause of a high incidence of chronic pyelonephritis among those with paraplegia or enlarged prostate, it remains unclear what invites infection in other types of obstructive uropathy, e.g., stricture or diverticula of ureter.

The following evidence supports infection as the cause of chronic pyelonephritis:

1. In animals such as rats and rabbits, renal lesions resembling chronic pyelonephritis have been produced by retrograde introduction of bacteria or by a single intravenous injection of certain bacteria.

2 It has been shown that pyelonephritic lesions can be readily produced in animals by obstructing the urinary outflow, an observation which reinforces the obstructive theory in humans.

3. Evolution of chronic pyelonephritis has been observed in children and adults after many years of acute urinary tract infection. A case history is presented later to illustrate this example. The progression of acute to chronic pyelonephritis has been observed especially in male paraplegics who have a history of repeated episodes of urinary tract infection.

4. The mere absence of a history of urinary tract infection in many patients does not rule out persistent asymptomatic bacteriuria.

5. Since there is a good correlation between bacteriuria and active flammation of the kidney, chronic pyelonephritis in many cases could be attributed to persistent asymptomatic bacteriuria.

Because of some disagreement concerning the specificity of the pathology of chronic pyelonephritis, it would be best if the relationships of this pathology with a variety of presumptive etiological factors were defined.

Three major factors have been universally implicated in the pathogenesis of chronic pyelonephritis: (1) obstruction of the urinary conduit, (2) asymptomatic bacteriuria, and (3) vesicoureteral reflux.

Obstruction of the Urinary Conduit

It has been stated that obstruction in any part of the urinary conduit is followed by infection. It is not understood how urinary tract obstruction increases the susceptibility of the renal parenchyma to infection and whether there is any evidence to support this notion. Experimental studies indicated that infection of the kidney occurred in 100% of rats and rabbits after the intravenous injection of lml. of Ringer's solution containing 3×10^8 *E. coli* following ligation of one of the ureters. The proportion of kidneys that develop infection in animals is directly related to the number of bacteria injected. This study has only slight relevance to humans since bacteremia proven by positive blood culture is found in no more

than 25% of patients with acute pyelonephritis. Although complete obstruction of the urinary tract increases the chance of infection markedly, it has been shown clearly that partial obstruction does not increase the chance of renal parenchymal infection in animals.

Although the exact mechanisms of renal infection after ureteral obstruction are still unclear, urinary stagnation secondary to obstructive passage is thought to be a major pathogenetic factor. It has been suggested that increased tissue pressure secondary to obstruction decreases the tissue resistance to infection. This idea is supported by the observation that occlusion of renal vein, which elevates intrarenal pressure, is followed by infection. The finding that, following intravenous injection of *E. coli,* bacterial multiplication occurred in the liver of guinea pigs with ligated bile duct reinforces the likelihood of infection in an obstructed kidney.

Although experimental studies are supportive of obstruction as primary to chronic pyelonephritis in humans, the observation does not explain the mode of infection above the obstruction. Also, the fact that the obstruction is by and large partial tends to clash with experimental study, in which complete obstruction only has been found to be conducive to renal infection.

It is even more intriguing to try to explain the spread of infected urine into the renal parenchyma, which causes renal parenchymal damage. The mechanism of progressive parenchymal damage, even if the infection is theoretically eradicated by antiinfective treatment, is also totally unclear. Observations in humans and animals (pigs) suggest that infected urine reaches the parenchyma by reflux from calyces to the tubules. Reflux of radioopaque material into renal parenchyma has been demonstrated during a voiding cystogram, especially in those with moderate to severe vesicoureteral reflux. Ransley and Risdon (1974) have demonstrated the anatomical basis for intrarenal reflux by studies in pigs. These authors have shown that the circular openings of the collecting ducts in the upper and lower polar papillae are unlikely to be occluded by rising intracalyceal pressure, in contrast to closure of slitlike duct orifices in the midzone papillae.

Several issues may be raised with regard to the mechanisms of progressive damage in the kidney (in the absence of a history of overt infection in the vast majority of the patients), some of which have the backing of sound reasoning while others lack real precision: (1) Radiological investigation demonstrated calyceal blunting and focal cortical atrophy in patients with a history of repeated episodes of urinary tract infection. (2) Persistent or repeated episodes of urinary tract infection are common in paraplegic patients who reveal progressive pyelonephritic changes. (3) Gradual decline in renal function in bilaterally obstructed kidneys and medullary cystic disease seems to coincide with progressive decreases of renal mass. The main challenge to the idea of persistent slow damage or repeated damages is the demonstration of chronic pyelonephritic kidneys in many patients who deny any history of urinary tract infection and fail to reveal any anatomical abnormality in the urinary tract which might have been associated with infection.

The next question concerns mechanisms of progressive damage to the kidney, the following having been proposed:

1. Many cases of suspected chronic pyelonephritis may have chronic interstitial nephritis of undetermined etiology.

2. Some cases have chronic pyelonephritis, especially those who provide a history of overt urinary tract infection. In these cases, although infection is eradicated by antibiotic therapy, continuation of residual infection in the renal parenchyma has been suggested. This idea is supported by the experimental observation of Kalmanson and associates (1965). These workers produced ascending acute pyelonephritis by introducing *Streptococcus faecalis* into the rat urinary bladder by urethral catheterization. The pathological, bacteriological, and immunological changes of this infection were studied for 40 weeks. Progressive changes of chronic pyelonephritis, cystitis, and ureteritis were found in 44 of 111 animals with negative bacteriological kidney cultures. Since the renal medulla and papilla are the sites of predilection for chronic pyelonephritis, antibiotics may not attain adequate concentration in these sites to kill the organisms. It has been suggested that in these sites the organisms are transformed into protoplasts or L forms in which state they live in the renal tissue for a long time. This is a distinct possibility because protoplastlike structures were found by EM study of renal tissue from one of our patients with chronic pyelonephritis (see later figures). However, there is no evidence to indicate that these L forms of bacteria or protoplasts are injurious to the tissues.

3. It has also been suggested that cystitis, which almost always accompanies pyelonephritis, damages the ureterovesical junction and causes ureterovesical reflux. Therefore, it is logical to conceive that after each episode of urinary tract infection more damage of the ureterovesical junction occurs. Thus, between episodes of overt infection, vesicoureteral reflux may perpetuate renal damage.

Sommers and associates (1964) have studied kidneys from Wistar rats after intravenous injection of 1 ml of 4.0×10^8 *Streptococcus faecalis* for a period of 3 to 21 weeks. In this study, the authors observed accelerated interstitial scar formation, glomerular sclerosis, and arteriolar sclerosis. These changes were not associated with the severe arterial thickening. However, these authors have suggested that repetitive infection for a period longer than 21 weeks may lead to the development of hypertensive vascular alterations.

4. It has also been proposed that, as in cirrhosis, the initiating factor (e.g., virus) need not persist for the destructive process to become progressive due to autoimmunity, altered vascularity, or other factors.

Asymptomatic Bacteriuria and Renal Disease

Urine formed by the kidney is usually bacteria free; also, when urine traverses the ureter it remains unpolluted by bacteria. The bladder urine may contain a few bacteria, but these are rapidly removed by the normal bladder. The urethral urine commonly contains bacteria. Since we know that bacteria are normally

present in urine, two questions have been posed: (1) what is a significant urine culture or significant bacteriuria, and (2) what is the relationship between bacteriuria and renal disease? Thus, a significant urine culture implies that repeated cultures will demonstrate the same species of bacteria, that the number of organisms per milliliter of urine is greater than the normal limit, which is set at 10^5/ml, and that the isolated bacteria have a pathogenic potentiality. A significant urine culture indicates a certain probability of a urinary tract infection but does not necessarily point toward an active disease process.

A positive urine culture in itself, regardless of the type of bacteria or the number of bacteria, does not necessarily indicate the presence of a urinary tract infection. Similarly, the reverse is also true, i.e., urine culture may be negative in the presence of active urinary tract infection, such as hematogenous pyelonephritis and pyelonephritis, in a kidney attached to a completely obstructed ureter. Too much emphasis must not be placed on the colony count of 100,000/ml as an absolute criterion. Thus Stamey and associates (1965) observed by culture studies of urine obtained via suprapubic aspiration that 33% of the patients had urinary tract infection although the colony count was less than 100,000/ml. In an autopsy study of 100 cases by McDonald and colleagues (1957), no correlation was found between bacteriuria and chronic or healed pyelonephritis, although a strong association between bacteriuria and acute pyelonephritis was obtained. A high percentage (22–24%) of pregnant women with asymptomatic bacteriuria have been found to develop acute pyelonephritis. Follow-up studies of women found to have bacteriuria during pregnancy have shown development of chronic pyelonephritis in 7 to 10% of these women. In British studies 25% of children with bacteriuria were found to have renal scars although the latter abnormality was of serious significance in no more than 10% of these children.

Bacteriuria is reportedly higher in patients with diabetes mellitus than in nondiabetic individuals. This bacteriuria may explain in part the high incidence of chronic pyelonephritis in diabetes mellitus. However, some studies have indicated that this may not hold true for male diabetics since no difference was observed in the incidence of bacteriuria between male diabetics and matched control subjects.

Vesicoureteral Reflux, Renal Scarring, and Chronic Pyelonephritis

Vesicoureteral reflux means retrograde flow of urine from the bladder to the ureter. This is an abnormal state and apparently the most common cause of chronic pyelonephritis in childhood. In one series, vesicoureteral reflux was found in 90% of 83 children who demonstrated unilateral or bilateral scarred kidneys. How vesicoureteral reflux produces chronic pyelonephritis remains an enigma. It has been assumed that infected bladder urine moves up the ureter and gains entrance into the renal parenchyma by intrarenal reflux, causing parenchymal damage and renal scarring. Refuting this assumption are the extensive observations of Smellie and Normand (1975). These authors failed to demonstrate appearance or extension of renal scar in a follow-up study for a period of 2 to 10

years of 150 children with vesicoureteral reflux, in whom urine was kept sterile by antimicrobial treatment. The infectious origin of renal scarring in childhood is contradicted by study in pigs, in which vesicoureteral reflux of sterile urine produced renal scarring similar to that observed in humans. In fact, scars were found at the sites of reflux, mostly in the upper pole of the kidney. This experimental observation suggests that renal scars are caused more by the direct effect of reflux than by infection. However, it is difficult to substantiate this experimental observation in humans, especially because pyuria and/or bacteriuria can be detected in many individuals with obstructive uropathy, vesicoureteral reflux, or diabetes mellitus who deny symptoms of urinary tract infection.

PERSPECTIVES OF CHRONIC PYELONEPHRITIS FROM THE STANDPOINT OF CLINICAL PRACTICE

This topic includes diagnosis and differential diagnosis, pathogenesis, relationship to clinical profiles, and prospects for transplantation.

Diagnosis and Differential Diagnosis

There are many obstacles in establishing the diagnosis of chronic pyelonephritis, including (1) lack of definite history of urinary tract infection in a substantial percentage of cases; (2) absence of pyuria and no bacterial growth in some cases; (3) rare incidence of microorganisms grown out of culture of renal tissues; (4) wide variations of the incidence of chronic pyelonephritis in autopsy studies as well as equal male-to-female ratio in sharp contrast to female preponderance for clinically evident acute pyelonephritis; (5) ethical problems pertaining to a single or serial renal biopsy in acute pyelonephritis, which have stood in the way of studying the natural history of the disease process; (6) false negative results in the biopsy studies caused by focal lesions, and especially location of the lesion at the upper pole; and (7) the fact that chronic interstitial nephritis resembling chronic pyelonephritis occurs in response to a variety of stimuli. Of these hindrances, the widely variable incidence (2.8–58%) reported in different series (Table 10-2) creates serious doubts as to the characterization of the histopathological features of chronic pyelonephritis.

Table 10-2
Incidence of Chronic Pyelonephritis

Authors	Year	Reported incidence (%)
Raaschou	1948	58
Kleeman *et al.*	1960	15
Kimmelstiel *et al.*	1961	2.8
Barnes *et al.*	1972	15
Freeman	1973	7

Relman (1972) has stated that chronic pyelonephritis is the most common primary renal disease in his experience, which is limited to adult medical renal clinics at university hospitals in Boston and Philadelphia. Prakash and associates (1962) from India have made a diagnosis of chronic pyelonephritis using the criteria of Kimmelstiel *et al.* (1961) in 26 of 105 renal biopsies in a period of 3 years. It is not yet known what has led to the wide difference in incidence; apparently it is due to the variations in the histopathological criteria used by different authors for the diagnosis of chronic pyelonephritis.

Criteria for the Histopathological Diagnosis of Chronic Pyelonephritis

The histopathological diagnosis of chronic pyelonephritis remains an enigma, although the criteria proposed by Kimmelstiel and associates (1961) are generally satisfactory: (1) infiltration of the interstitium by pleomorphic cells containing lymphocytes, plasma cells, and monocytes; (2) the presence of polymorphonuclear leukocytes in addition to the chronic inflammatory cells in the interstitium; (3) thyroidlike appearance of the tubules; (4) coarse scars; (5) U-shaped surface scars; and (6) exclusion of other causes of chronic interstitial nephritis.

Most investigators are inclined to believe that although interstitial infiltration by lymphocytes alone is of slight or no diagnostic value, the presence of a combination of lymphocytes and plasma cells in the interstitium constitutes good evidence for bacterial infection, i.e., chronic (bacterial) pyelonephritis. According to many observers, this proposition is reinforced by the demonstration of neutrophil leukocytes in the interstitium or tubular casts impregnated with neutrophil leukocytes. Morphology of glomeruli and arterial vessels is apparently of no value in distinguishing chronic pyelonephritis from other types of chronic interstitial nephritis. However, changes in these two component parts would assist in separating glomerular and vascular diseases from chronic pyelonephritis. There are two conditions which mimic chronic pyelonephritis: focal glomerular sclerosis and diabetic kidney. The differential diagnosis is discussed later.

Notwithstanding the difficulties in confirming a diagnosis of chronic pyelonephritis, Sommers (1977) firmly believes that chronic pyelonephritis is a distinct pathological state separate from chronic interstitial nephritis. The author is in agreement with Sommers, and cites examples to support the entity of chronic pyelonephritis.

Patient #1. L.B., a 53-year-old white male, was admitted to the Oklahoma City VA Hospital in January 1974, with a 5-day history of dyspnea and pedal edema. He had three hospital admissions in 1973, for acute myocardial infarction, acute urinary retention, and cardiac arrhythmia. On admission, he showed signs of congestive cardiac failure with mild respiratory distress, neck vein distension, diffuse expiratory rhonchi, tender hepatomegaly (14 cm), and pedal edema. His blood pressure was 120/80 mm Hg. (For laboratory information see Table 7-7.) He had congestive changes bilaterally on a chest x-ray, old anteroseptal myocardial infarction by ECG, cortical atrophy, as well as calyceal clubbing

on left renal arteriography and patency of the renal veins on renal venography. Bilateral retrograde pyelography revealed clubbing of the lower pole calyx and a "ring shadow" (a characteristic radiological sign of papillary necrosis) in the upper pole calyx of the left kidney, faintly visualized calyces in the right kidney, asymmetry in size of the kidneys, with the left smaller than the right by more than 1 cm, and a stone in the left ureter. Symptoms and chest x-ray findings cleared with digitalization and diuretic therapy. A percutaneous right renal biopsy was performed. Renal tissue was studied by light, electron, and immunofluorescence microscopy (LM, EM, and IFM, respectively). LM revealed a total of 20 glomeruli, of which 18 were normal and two were hyalinized. The consistent changes were periglomerular fibrosis, a scarcity of tubules with the remainder atrophic and dilated, and focal interstitial infiltrates of lymphocytes and neutrophils. Some dilated tubules contained necrotic material impregnated with neutrophil leukocytes and lymphocytes (Fig. 10-1). Small arterioles were observed and appeared to be normal. Gram staining of the renal tissue demonstrated gram-positive organisms.

EM of two glomeruli showed fusion of foot processes in most of the capillaries (see Fig. 7-37). The glomerular basement membranes in some capillaries were slightly irregular and tortuous. Endothelial cells in some capillaries were slightly enlarged and contained vacuoles. There was a remarkable absence of hypercellularity and electron-dense deposits. EM of tubules and interstitium revealed remarkable changes characterized by massive

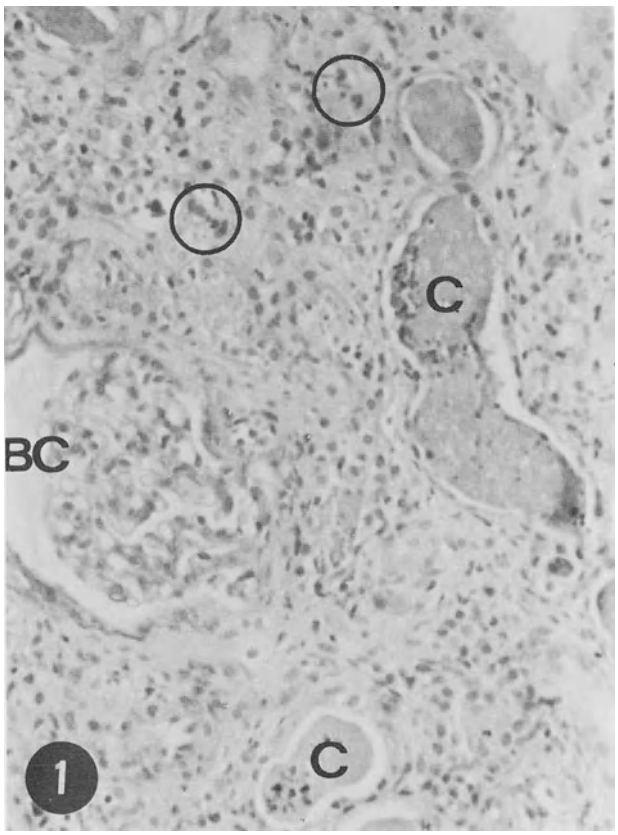

Fig. 10-1. A glomerulus with normal tufts and intracapsular fibrosis, i.e., fibrotic zone inside the Bowman's capsule (BC), conspicuous dearth of tubules, dilated tubules with absent or flattened epithelium and containing casts, some of which are impregnated with neutrophil leukocytes. Two dilated tubules filled with leukocytic casts (C) are shown in this figure. Interstitium is fibrotic and infiltrated with lymphocytes, plasma cells, and neutrophil leukocytes (circles) (H & E, ×80).

infiltration of the tubules and interstitium by lymphocytes (Fig. 10-2). Lymphocytes were found between the basement membrane and cells of the tubule (Fig. 10-3). Some of the tubules were intact, others were atrophic, and still others were disorganized. Occasional tubules contained a cast impregnated with degenerated neutrophil leukocyte (Fig. 10-3). Bacterial (protoplast) types of structures were found inside the tubules (Fig. 10-4) and in the interstitium (Fig. 10-5). These structures were similar to those demonstrated by Dublin and Shimamura (1975) in experimental acute pyelonephritis. IFM of renal tissue, stained with fluorescein-conjugated antisera against human IgG, IgA, IgM, IgE, third component of complement (C3), and fibrinogen, was negative. Becasue of the EM diagnosis of lipoid nephrosis, negative staining for all immunoglobulins, and persistent massive proteinuria (1 month after discharge, 24 hr proteinuria was 17.9 g), the patient was placed on oral prednisone 80 mg daily. After 2 weeks, his 24-hr proteinuria fell to 2.4 g. After 4 weeks of

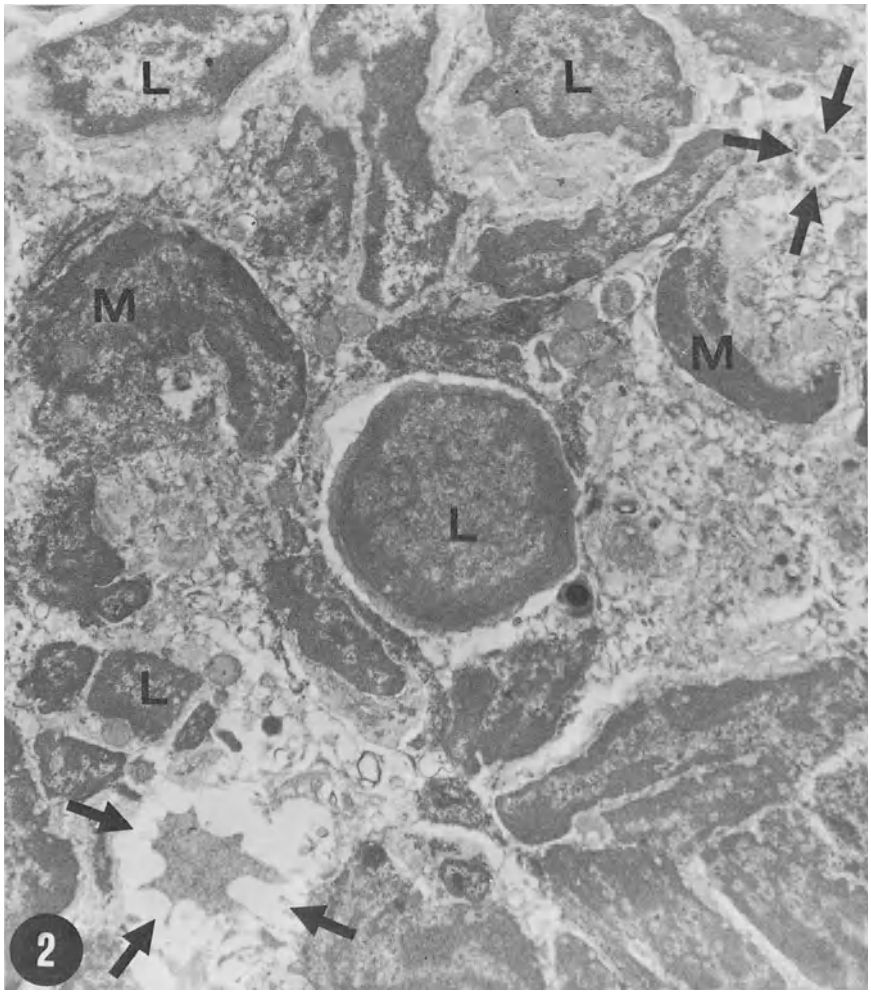

Fig. 10-2. Infiltration of the interstitium by lymphocytes (L) of variable size, monocytes (M), and bacterialike structures (arrows) (UA + LC, ×10,000).

treatment, his laboratory studies revealed proteinuria 1.1 g/24 hr, serum urea nitrogen 54 mg/100 ml, and serum albumin 4.2 g/100 ml. He expired suddenly before the next visit and no autopsy was performed.

Comments. This patient presented several important aspects in support of a diagnosis of chronic pyelonephritis: (1) pyuria and significant urine culture; (2) radiological evidence of left obstructive uropathy; (3) radiological signs of chronic pyelonephritis in the left kidney; (4) light microscopy histopathology consistent with chronic pyelonephritis in the right kidney; (5) gram-positive organisms in the renal tissue; (6) electron microscopy demonstrating abundant lymphocytes, and, in fact, replacement of a large part of the renal tissue by lymphocytes; and (7) and normal blood pressure and essentially normal renal arterioles.

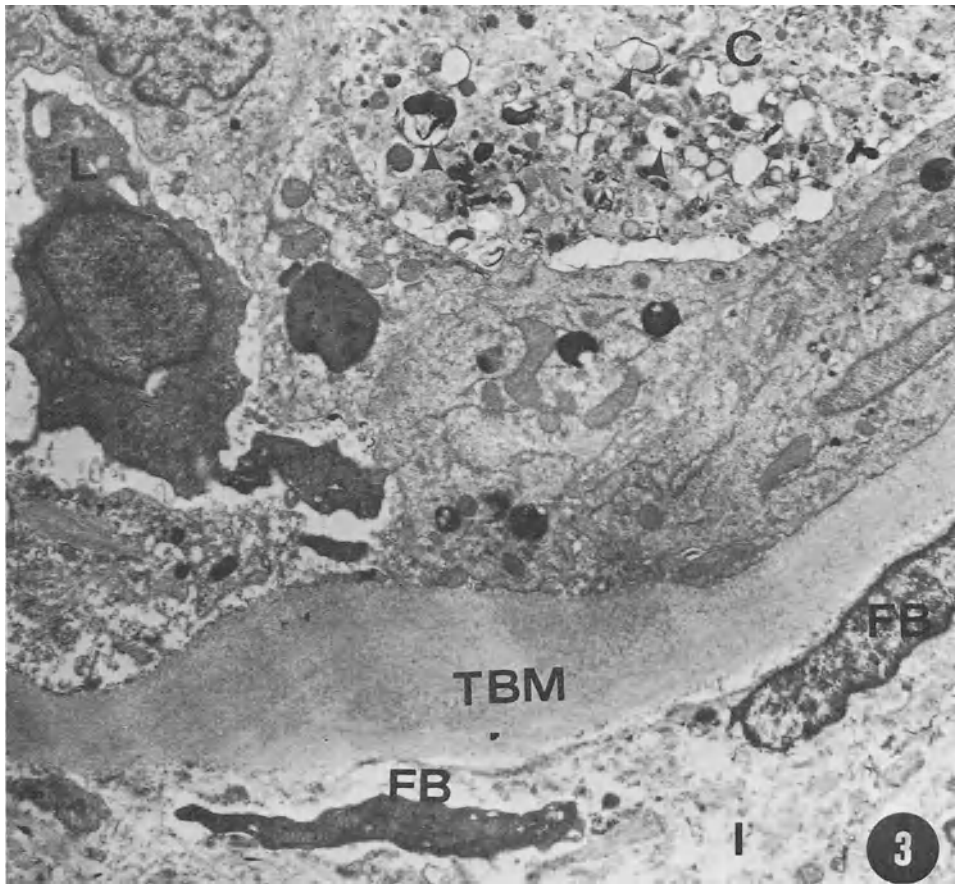

Fig. 10-3. Invasion of the tubule by a lymphocyte (L) and occlusion of the lumen by a cast (C) containing a degranulated leukocyte. The vacuoles (arrowheads) represent degenerated neutrophil granules. The tubular basement membrane (TBM) is moderately thickened and the tubule as a whole is distorted. Interstitium (I) is slightly edematous and shows fibroblasts (FB) (UA + LC, ×21,000).

Therefore, this study suggests that (1) even when the obstruction is unilateral, chronic pyelonephritis tends to be bilateral; (2) renal biopsy may prove to be a useful aid in cases of suspected unilateral pyelonephritis; and (3) there is a need of EM study to document lymphocytic (and plasma cellular) abundance and bacillary bodies (Figs. 10-2 to 10-5) in chronic pyelonephritis.

Patient #2. J.O., a 66-year-old white male, was referred to the Renal Division of the University of Oklahoma Health Sciences Center for evaluation of anemia, proteinuria, microhematuria, and azotemia. He admitted to frequency, nocturia, urgency, hesitancy, and urinary dribbling for several months. Physical examination revealed pallor, bilateral basilar rhonchi and rales, mild pitting edema, and a blood pressure of 168/82 mm Hg. (For laboratory data, see Table 7-7.) An infusion pyelogram showed a faint pelvicalyceal system in bilaterally small kidneys. Residual postvoiding volume was 100 ml. The patient had a percutaneous right renal biopsy and the renal tissue was studied by LM and EM. LM revealed a total of 10 glomeruli, of which one was hyalinized and the remaining nine were normal with the exception of a slightly increased mesangial matrix in one-third of them. Periglomerular fibrosis, dilated tubules filled with casts, focal atrophy of tubules, patchy cellular infiltrates, and thickening of arterial vessels were observed. EM of glomeruli showed generalized fusion of epithelial foot processes in all glomerular capillaries with slight irregularity on the endothelial side of the basement membrane in some capillaries. EM of large arterioles revealed thickening of the endothelial basement membrane, exces-

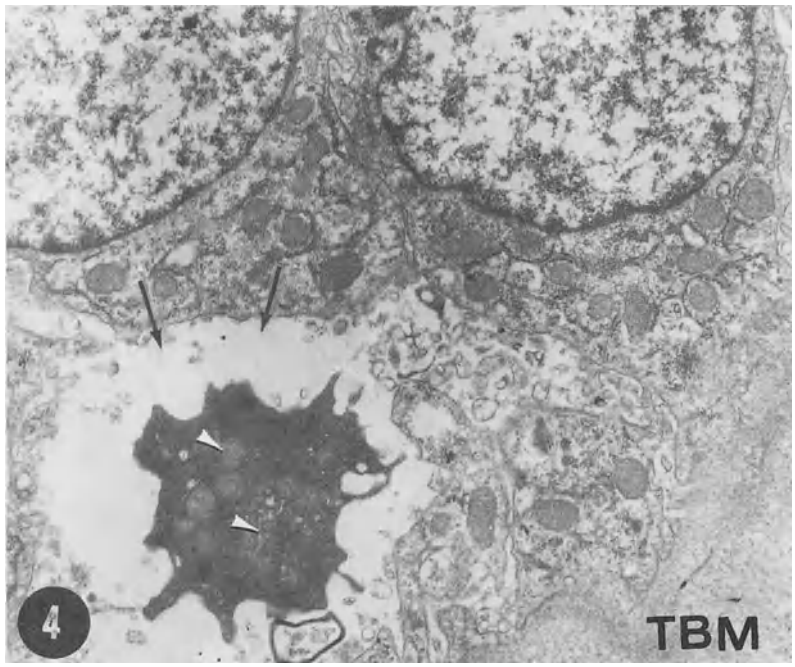

Fig. 10-4. Invasion of the tubule by a macrophage containing bacterialike structures (arrowheads). There is a conspicuous halo around the macrophage (arrows). The basement membrane of the tubule (TBM) is necrotic and the tubule is somewhat disorganized (UA + LC, ×18,000).

sive basement membrane materials, and atrophy of some smooth muscle cells. The patient was lost to further follow-up.

Patient #3. A.A., a 14-year-old black female, was seen for the first time at Oklahoma Children's Memorial Hospital in May 1972 for anasarca of 1 week's duration. She gave a history of eczematoid dermatitis. The physical examination showed anasarca, an eczematoid rash on the forehead and posterior part of the neck, pharyngeal irritation, and a blood pressure of 130/100 mm Hg. Her throat culture grew group A betahemolytic streptococci. Her ASO titer and serum C3 were normal. A drip infusion pyelogram revealed a poorly visualized nephrogram and no pyelogram. (The remaining laboratory values are shown in Table 7-7.) A percutaneous right renal biopsy was performed and the tissue was fixed for LM and EM. LM revealed a few glomeruli, all of which were normal, a mild reduction in the total number of tubules, with atrophy and dilatation of a few tubules each (Fig. 10-6). She was treated with salt restriction and diuretics.

Because of persistent massive proteinuria, a second renal biopsy was performed 3 months after the first biopsy and the tissue was studied by LM, EM, and IFM. LM

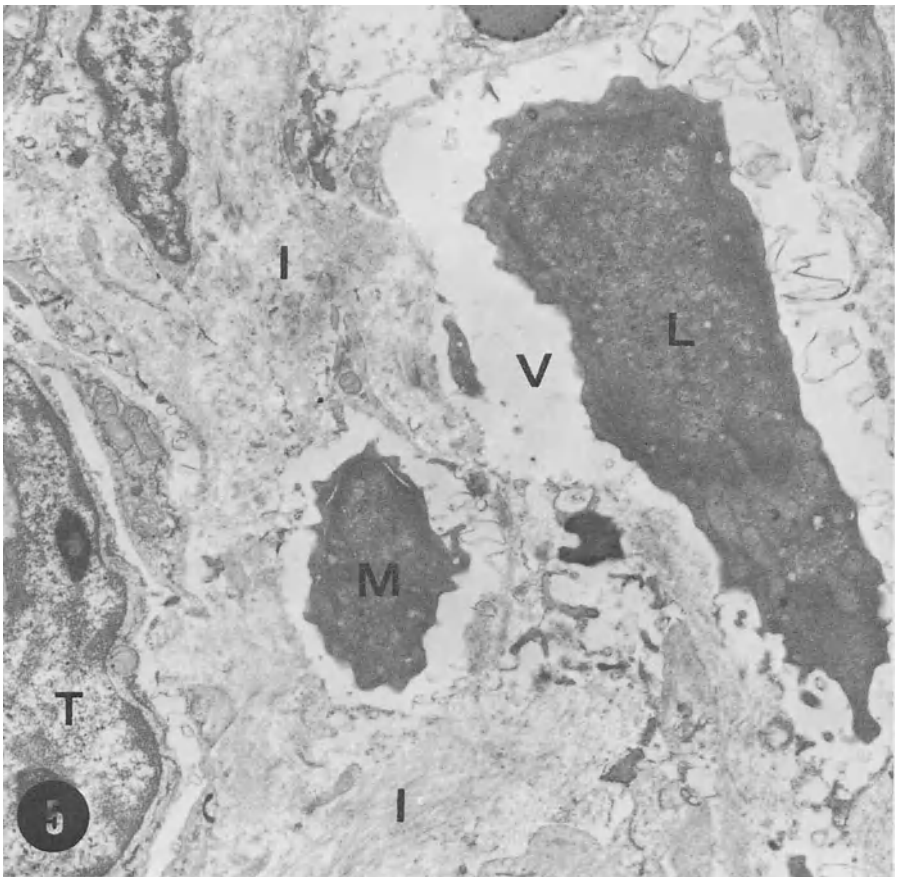

Fig. 10-5. Note the organisms inside and the halo around a macrophage (M) in the fibrotic interstitium (I), a lymphocyte (L) in the vein (V), and a disrupted tubule (T) (UA + LC, ×16,000).

findings were not different from those in the first biopsy (Fig. 10-7). EM of a glomerulus revealed generalized fusion of epithelial foot processes with a normal appearance of glomerular basement membrane, atrophy of tubules, and interstitial infiltration by lymphocytes and plasma cells. IFM was negative for all immunoglobulins, C3, and fibrinogen. She received a course of prednisone orally, 60 mg daily for 4 weeks, followed by tapering doses for another 4 weeks. She continued to have edema and hypertension and her renal function progressively deteriorated. Twenty-four months after the first visit, her blood pressure was 190/130 mm Hg and blood urea nitrogen was 212 mg/100 ml. At this time, she was started on maintenance hemodialysis.

Thirty months after the first visit, she underwent bilateral nephrectomy; the two kidneys weighed 44 and 43 g each (normal weights for age, 100 g each). They were deep gray to brown and included a small amount of peripelvic fat. The renal tissues were fixed for LM, EM, and IFM studies. LM demonstrated normal glomeruli, a marked dearth in the number of tubules with dilatation of residual tubules containing proteinaceous material, widespread interstitial infiltrates, and patchy infiltration with large collections of inflammatory cells (Figs. 10-8 and 10-9), widespread fibrosis, and slightly thickened arterial vessels. EM of three glomeruli revealed fusion of epithelial foot precesses in most capillaries with the presence of a few discrete foot processes in some of these capillaries (Fig. 10-10). Except for slight irregularity on the endothelial surface, the glomerular basement membranes were essentially normal. There was an absence of hypercellularity or electron-dense deposits in the glomerular capillaries. The IFM was negative for all immunoglobulins, C3 and fibrinogen.

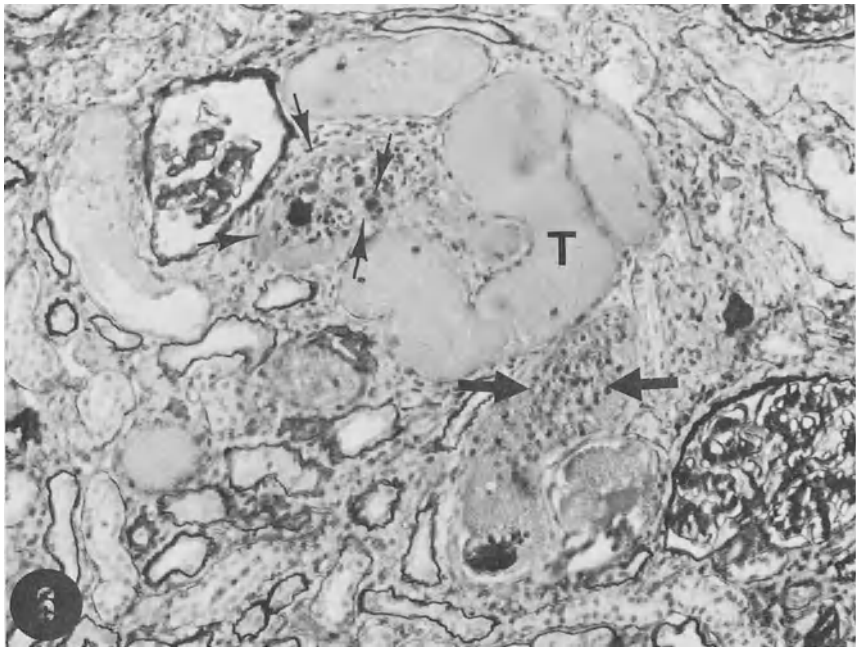

Fig. 10-6. This micrograph shows normal glomeruli and a small number of tubules, of which most are atrophic and some are dilated and filled with casts to produce a thyroidlike appearance (T). In some tubules casts are impregnated with neutrophil leukocytes (opposing arrows) (PAMS, ×80). From the first biopsy of patient #3.

Comments. This patient presents many features in favor of chronic pyelonephritis: (1) markedly small size of the kidneys, (2) marked decrease in the population of the tubules in a period of 30 months, (3) conspicuous focal infiltration of inflammatory cells, (4) development of renal failure even though the glomeruli appear normal by LM, and (5) pyuria.

Differential Diagnosis

1. The histopathological picture of chronic pyelonephritis may be misinterpreted as that of focal glomerular sclerosis. The latter can be excluded by the absence of segmental glomerular sclerosis and hyalinosis (LM study), absence of the basement membrane abnormalities, excessive basement membrane-like materials, and electron-dense deposits in the glomeruli (EM study). Infiltration of the interstitium by lymphocytes and plasma cells should not be considered as a

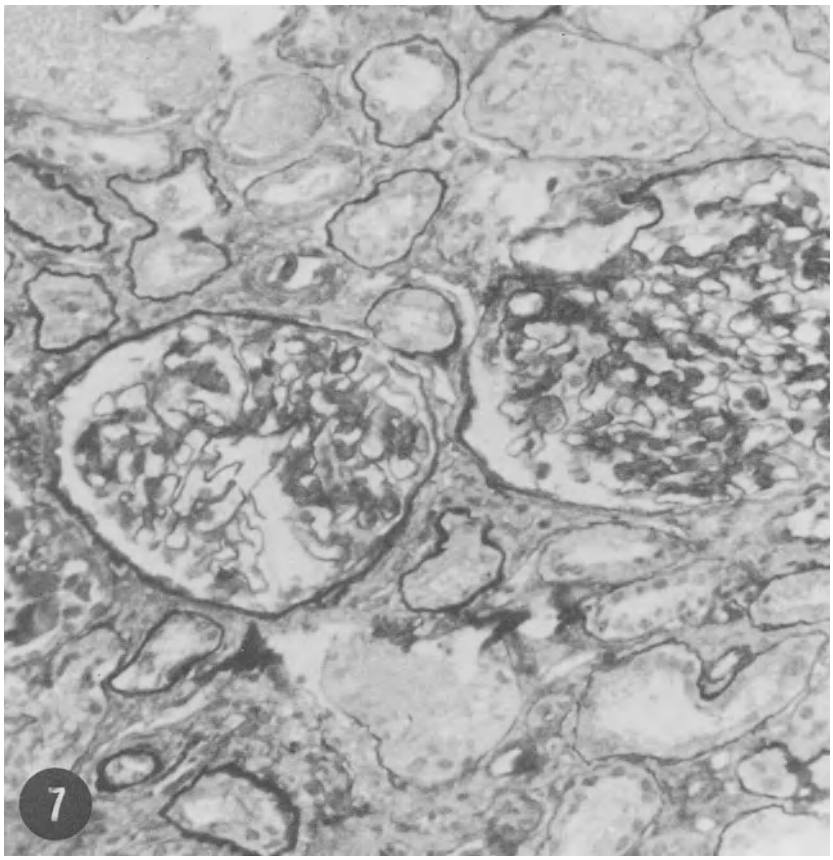

Fig. 10-7. Note normal glomeruli, reduced number of tubules, some of which are atrophic, and mild interstitial fibrosis (PAS, ×120). From the second biopsy of patient #3.

dependable criterion to distinguish chronic pyelonephritis from focal glomerular sclerosis because of identical interstitial infiltration observed in the latter pathological state (Fig. 10-11).

2. Acute and chronic pyelonephritis may coexist with diabetic glomerulosclerosis and arteriosclerosis and arteriolosclerosis. In cases with diabetic glomerulosclerosis alone, tubular atrophy and dilatation along with interstitial infiltrates may be observed. In such instances, careful observation may disclose nodular or diffuse diabetic glomerulosclerosis. The temptation to distinguish

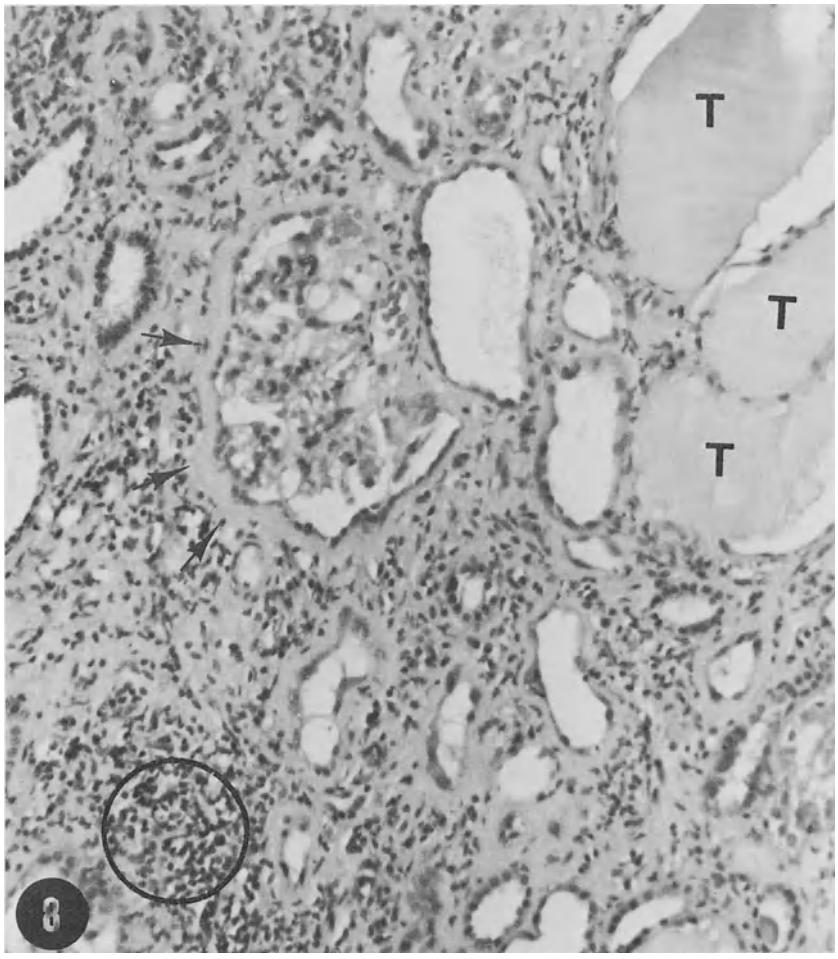

Fig. 10-8. Marked scarcity of tubules with the remaining widely dilated and filled with casts to produce a thyroidlike appearance (T). This along with interstitial fibrosis and focal collections of inflammatory cells (circle) constitutes a conspicuous feature of this biopsy. The glomerulus reveals pericapsular fibrosis (arrows) but otherwise appears normal (H & E, ×120). From the nephrectomy specimen of patient #3.

primary chronic pyelonephritis from that associated with diabetes mellitus may be a futile gesture.

The exact cause of the high incidence of acute and chronic pyelonephritis in diabetes mellitus is unknown. Does sclerosis of the glomeruli, tubule, and arterial vessels render the kidney susceptible to infection? This is an unlikely possibility because similar sclerosis in focal glomerular sclerosis does not predispose to infection. Two distinct changes in the kidney have some bearing: (a) thickening of peritubular capillaries with consequent ischemia of the tubules (for further discussion, see Renal Involvement in Diabetes Mellitus, Chapter 7) and (b) autonomic neuropathy with decreased contractility of urinary bladder. This then leads to increased residual urine, bacterial multiplication, cystitis, vesicoureteral reflux, and renal damage. Acute and chronic pyelonephritis in diabetes mellitus

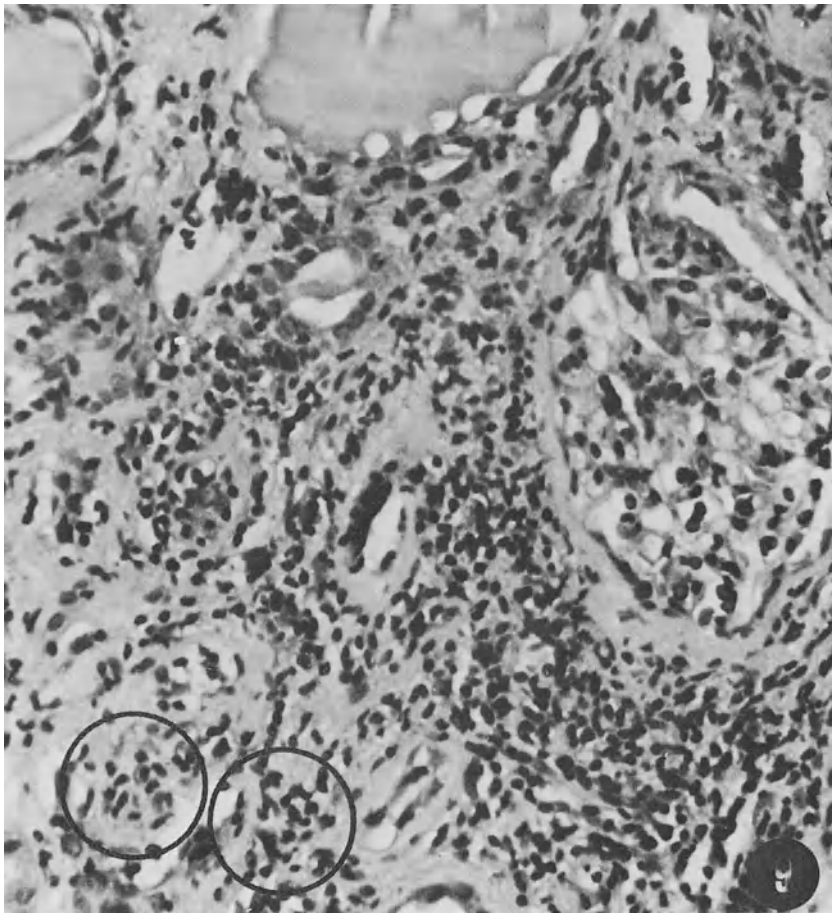

Fig. 10-9. Marked pleomorphic cellular infiltration in the interstitium. Neutrophil leukocytes (circles) are not uncommon. Note normal glomerulus and a widely dilated tubule filled with a cast (H & E, ×320). From the nephrectomy specimen of patient #3.

have certain clinical importance. There is some agreement that intermittent (i.e, 1 week once a month) treatment with antibiotics is useful in retarding the progression of renal failure.

UNRESOLVED QUESTIONS

Exacting Diagnosis

There are many arguments against isolation of chronic pyelonephritis as an entity separate from chronic interstitial nephritis. In view of the disagreement,

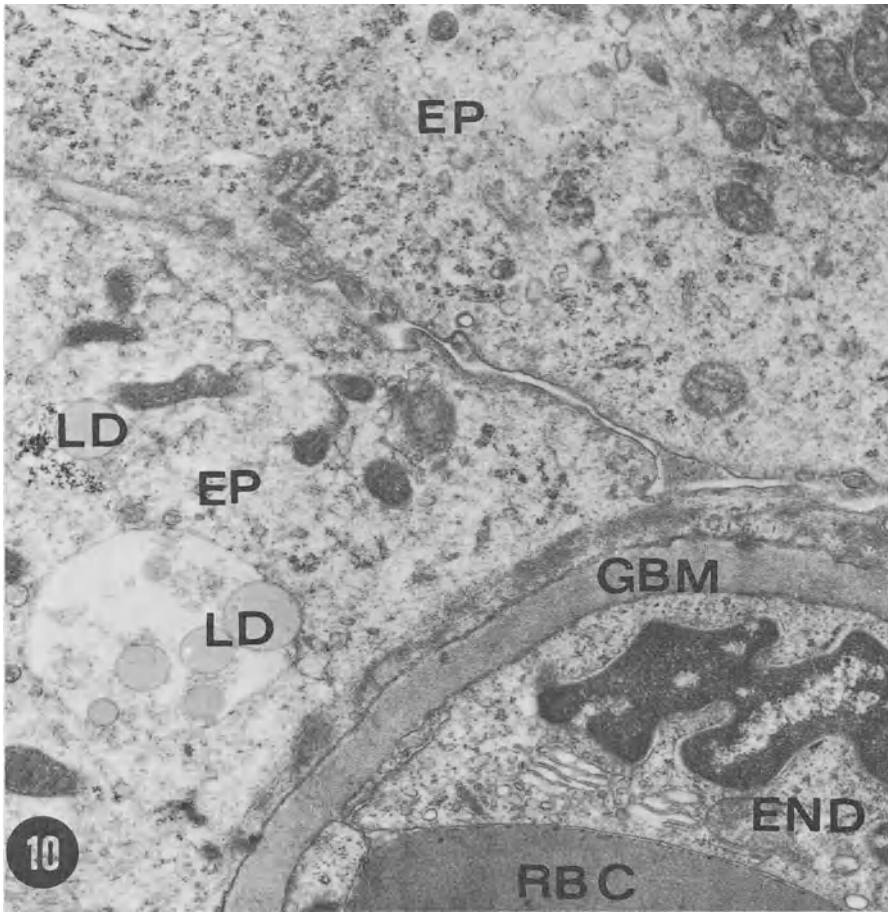

Fig. 10-10. This glomerular capillary reveals complete fusion of foot processes and epithelial cells (EP). The EP is active, and is characterized by abundant ribosomes, rough-surfaced endoplasmic reticulum, and many mitochondria. Lipid droplets (LD) are seen in the EP. The glomerular basement membrane (GBM) is normal. Endothelial cell (END) of the glomerular capillary and a red blood cell (RBC) are shown (UA + LC, ×18,000).

a proposition can be made that may arbitrate the argument between those who favor and those who are against the separation. The term *chronic pyelonephritis,* in my own opinion, denotes a clinical condition characterized by a history of repeated episodes of urinary tract infection, pyuria, slight proteinuria, leukocyte urinary casts, significant urine culture along with radiological signs of involvement of the pelvicalyceal system (clubbing of a calyx or calyces, rings sign, etc.), and focal cortical scarring. The term *chronic interstitial nephritis* should be reserved as a descriptive term for the histopathological changes characterized by

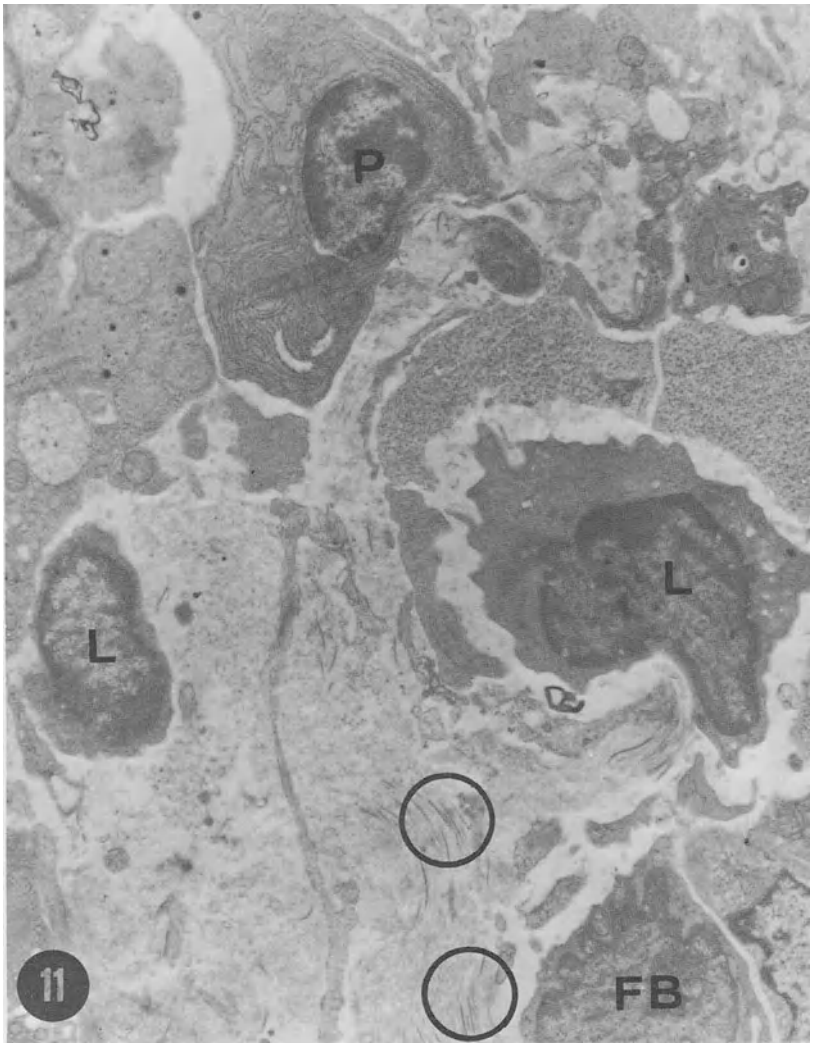

Fig. 10-11. A conspicuous interstitium due to infiltration by lymphocytes (L), plasma cell (P), fibroblasts (FB), and collagen fibers (circles). From a patient with focal glomerular sclerosis. These EM findings resemble those observed in chronic pyelonephritis (UA + LC, ×12,000).

interstitial cellular infiltrates, tubular atrophy and dilatation, and the presence of casts within the tubules with or without hyalinization of the glomeruli and with or without changes in the arterial vessels. The latter is in accordance with the statement of Heptinstall (1976).

It seems unjustified to predict the clinical state on the basis of histopathological changes, i.e., the patient has chronic interstitial nephritis of bacterial origin (chronic pyelonephritis) or abacterial chronic interstitial nephritis of undetermined etiology. Rather, it would be wise to assess the functional status of the kidney based on tubular mass and glomerular, vascular, and interstitial changes. A histopathological diagnosis of chronic pyelonephritis (bacterial pyelonephritis) becomes certain if organisms are found on gram staining of the tissue, if pathogens grow on culture of the tissue, and if abundant lymphocytes, plasma cells, and neutrophil leukocytes along with bacteria (protoplast)-like structures are confirmed to be present in the renal tissue studied by EM.

Complications of Chronic Pyelonephritis

Three important complications deserve a brief discussion within the limits of this book: hypertension, uremia, and nephrotic syndrome.

Hypertension

Although a variable incidence of hypertension has been reported in chronic pyelonephritis, the relationship between the two conditions has not been established because a patient with essential hypertension may develop chronic pyelonephritis, and vice versa, and renal histopathology of essential malignant hypertension resembles that of malignant hypertension secondary to chronic pyelonephritis. Therefore, renal histopathological study alone is unable to determine the sequence of events between hypertension and chronic pyelonephritis. In addition, hypertension appears to be more common in chronic pyelonephritis with no evidence of urinary tract obstruction than in that associated with unilateral obstructive uropathy. This dissociation can be observed in the three patients presented in this chapter. Patients #1 and #2 had urinary tract obstruction and normal or near-normal blood pressure, whereas patient #3, with no evidence of obstructive uropathy, had mild hypertension at the time of admission and developed severe hypertension 24 months later. Bengtsson and associates (1968) reported that hypertension doubled after a mean follow-up period of 5.3 years. In a London university hospital study 20% of the children with chronic pyelonephritis were found to have hypertension. Thus, the widely variable incidence of hypertension (20–70%) appears to be caused by (1) the overestimation of the histopathological diagnosis of chronic pyelonephritis, (2) the difficult differentiation between hypertensive renal lesions and chronic pyelonephritis on the basis of histopathology alone, and (3) the difficult determination of the relationship between hypertension and chronic pyelonephritis in mild or asymptomatic cases.

With respect to the pathogenesis of hypertension in chronic pyelonephritis

opinions differ between intrarenal arterial obstruction with activation of renin–angiotensin mechanism and renoprival hypertension. The fact that hypertension with unilateral pyelonephritis is sometimes cured by a nephrectomy provides evidence of a causal relationship between hypertension and chronic pyelonephritis. The renin–angiotensin mechanism is an unlikely possibility since no alteration was found in the size and number of juxtaglomerular cells of the pyelonephritic kidneys. Furthermore, unilateral nephrectomy does not ameliorate hypertension in most instances. Sommers and associates (1962) found greater damage and loss of the proximal tubules in kidneys with both hypertension and chronic pyelonephritis than those with chronic pyelonephritis alone. These authors have conceded on the basis of histopathological evaluation that hypertension in chronic pyelonephritis may be regarded as an essentially renoprival process. I am in agreement with them because of my experience in the study of renal papillary interstitial cells in normotensive and hypertensive states.

Uremia

Despite normal morphology of most of the glomeruli and the arterial vessels, progressive renal failure occurs in many cases of chronic pyelonephritis. The exact incidence of uremia in chronic pyelonephritis is unknown. This lack of knowledge is caused in part by the "end stage appearance" of the renal morphology in some of these cases. In addition, hypertension has been implicated as the cause of uremia in a substantial percentage of cases of chronic pyelonephritis. Even if hypertension is a common accompaniment of chronic pyelonephritis complicated by uremia, small arterial vessels of the kidney may not demonstrate severe damage to account for uremia. This has been exemplified in patient #3. It was stated in Chapter 9 that despite severe arteriologlomerular changes in spontaneously hypertensive rats, renal failure was not observed in these rats. Therefore, impairment of renal function found in all the patients presented in this chapter and in similar patients reported by others can be more safely attributed to tubulointerstitial changes than to glomerular or vascular pathology. This statement is in accordance with the observation of other investigators. Thus, Risdon and colleagues (1968) have obtained the best correlation between tubulointerstitial changes and plasma creatinine concentration or creatinine clearance from an analysis in glomerular diseases.

Nephrotic Syndrome

For a discussion of nephrotic syndrome in chronic interstitial nephritis, see pages 225–229.

Pathogenesis of Chronic Pyelonephritis

Although a bacterial infection is suspected in almost every instance of chronic pyelonephritis, this is documented in only a small percentage of cases.

This clinicopathological disparity may mean that (1) infection is not the cause of most cases of chronic pyelonephritis, (2) pathogens may initiate the renal damage and then disappear, and (3) pathogens may cause the renal lesions of chronic pyelonephritis via an indirect route, i.e., antibody mechanism. The last hypothesis seems to be promising, especially in view of negative bacteriological evidence in clinically and histologically suspected cases of chronic pyelonephritis. Nimmich and associates (1976) in Germany measured antibody titers (indirect immunofluorescence and passive hemagglutination techniques) in patients with clinical diagnosis of chronic pyelonephritis. The authors found elevated antibody titers in a high percentage of patients with a clinical diagnosis of chronic pyelonephritis as compared to the values in healthy subjects. Consistently high antibody titers have been found in the absence of bacteriuria. The authors postulated that high antibody titers in the apparent absence of the pathogens in the urine must be caused by continuing antigenic stimulus resulting from the presence of either viable organisms in small closed foci or dead bacteria remaining in the kidney. It was concluded that measurement of antibody titers by the indirect immunofluorescence technique is a reliable tool for the diagnosis of chronic pyelonephritis.

Because high titers of antibody are found in clinically evident chronic pyelonephritis, an antigen–antibody complex formation and its localization in the renal tubules can be proposed. This type of immune complex may cause disappearance of pathogens from the renal tissue and yet continue to have damaging effect on the renal tissue. The evidence in favor of this hypothesis includes that of Aoki and associates (1969) who demonstrated *E. coli* antigen in six of seven kidney specimens from patients with abacterial pyelonephritis. Also to be considered are the experimental observations of Steblay and Rudofsky (1971), which include the following: (1) Guinea pigs injected with rabbit renal tubular basement membrane and Freund's adjuvant developed cortical tubular disease with glycosuria, azotemia, and proteinuria. Some of the animals died in acute renal failure, whereas others developed chronic tubulointerstitial disease. (2) Anti-TBM (anti-tubular basement membrane) autoantibodies were demonstrated in the serum and kidney elutes of guinea pigs with tubular disease. These antibodies reacted specifically with cortical TBM, but not with glomerular basement membrane (GBM), Bowman's capsule, or medullary TBM. (3) The characteristic linear staining for IgG along the TBM *in vitro*.

The relevance of anti-TBM antibodies in human diseases is as follows:

1. Anti-TBM antibodies have been demonstrated in rapidly progressive glomerulonephritis, a disease characterized by marked tubular atrophy and interstitial fibrosis in addition to the glomerular changes.
2. Anti-TBM antibodies have been reported in patients with renal allografts.
3. Anti-TBM antibodies in the serum of a patient with methicillin-associated acute interstitial nephritis are worth mentioning. A methicillin metabolite (dimethoxyphenylpenicillolyl) and IgG and C3 were bound to the tubular basement

membrane. Circulating antibodies were found that reacted with tubular basement membranes of normal human and monkey kidneys.

It is difficult to evaluate whether the anti-TBM antibodies are primary to tubulointerstitial changes, or if they are produced and deposited secondarily on the damaged tubules. Although there are reports to indicate that anti-TBM antibodies have been found in tubulointerstitial nephritis, just as high antibody titers have been found in chronic pyelonephritis, the evidence is not yet strong enough to implicate the antibody mechanism in chronic pyelonephritis in humans. The idea of delayed hypersensitivity to exogenous antigens, i.e., bacterial infection, manifested by infiltration of the kidney with lymphocytes and plasma cells, has reasonable promise.

Prospects for Renal Transplantation

The treatment of end-stage renal disease caused by chronic pyelonephritis is not unlike that of other forms of end-stage renal disease. Renal transplantation must be preceded by bilateral nephrectomies, or nephroureterectomies if refluxing ureters are present. The transplanted graft ureter may be emptied into an ileal conduit if the lower tract is unserviceable. In general, these patients have a greater likelihood for recurrence of pyelonephritis in the allograft, so that frequent urine cultures are mandatory, and antibacterial urinary prophylaxis may need to be considered. The long-term results of such allografts do not appear to differ significantly from those in which the allograft was necessitated by preceding glomerulonephritis.

SUMMARY

1. In the practice of nephrology, chronic pyelonephritis is one of the most perplexing subjects because of ambiguities concerning pathogenesis, the lack of absolute histopathological features, and sampling errors of the biopsy specimens used to diagnose the condition in the living state.

2. Although bacterial infection is almost always suspected in chronic pyelonephritis, seldom has the presence of infection been documented in the renal tissue. This negative evidence seriously compromises the distinct entity of chronic pyelonephritis. In fact, most observers believe that it is unsafe to try to separate chronic pyelonephritis from chronic interstitial nephritis.

3. Chronic pyelonephritis in adults is, by and large, secondary to some form of urinary tract obstruction. In contrast, chronic pyelonephritis in children is often associated with vesicoureteral reflux.

4. It is undoubtedly true that infection occurs in the obstructed kidney. This has been proven in both humans and animals, but the factor facilitating

entry of infected urine into the renal parenchymatous tissue is unknown. Intrarenal reflux from calyces to the tubules has been demonstrated during voiding cystograms and has been proposed to be a logical mechanism. The question remains as to whether it is the reflux, the infection, or both that produce parenchymatous damage. In animals, vesicoureteral reflux of sterile urine produces the same type of renal scarring as is observed in humans with chronic pyelonephritis.

5. Significant urine culture means a bacterial colony count of $>10^5$/ml, growth of the same species of bacteria in repeated cultures, and isolated bacteria possessing a pathogenic potentiality. A significant urine culture indicates a certain probability of a urinary tract infection, but does not necessarily point toward an active disease process.

6. There are many obstacles to establishing a diagnosis of chronic pyelonephritis. One of them concerns the absence of a history of urinary tract infection in the vast majority of cases. Asymptomatic bacteriuria and pyuria may account for latent chronic pyelonephritis in many cases.

7. A histopathological diagnosis of chronic pyelonephritis can be made on the basis of (1) interstitial infiltration by lymphocytes, plasma cells, and neutrophil leukocytes; (2) thyroidlike appearance of the tubules; and (3) exclusion of other causes of chronic interstitial nephritis. In view of serious arguments concerning the validity of these diagnostic criteria, it may be stated that diagnosis of chronic pyelonephritis becomes certain if these changes are accompanied by (a) organisms found on gram staining of the tissue or (b) pathogens isolated on culture of the tissue.

8. Negative evidence of the presence of organisms in the renal tissue cannot rule out the possibility that renal damage has been induced by pathogens. Progressive damage may occur as a result of repeated episodes, many of which may not be clinically overt. An immunological mechanism involving anti-TBM antibody has been postulated. This antibody, akin to anti-GBM antibody, is suspected to cause progressive tubulointerstitial disease, changes similarly observed in rapidly progressive glomerulonephritis.

9. A variable incidence of hypertension and uremia has been found in chronic pyelonephritis. The relationship of chronic pyelonephritis to each of the two complications and the potentiating effect between the two is undetermined.

10. Nephrotic syndrome occurs in a small percentage of cases of chronic pyelonephritis. Although heavy proteinuria occurs, the mechanisms have remained unknown until recently. Electron microscopy study has resolved that foot process fusion disease or lipoid nephrosis may be the cause of heavy proteinuria or nephrotic syndrome in chronic pyelonephritis.

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11 Syndrome of Microscopic Hematuria, Asymptomatic Proteinuria, and "Focal Glomerulonephritis"

INTRODUCTION

Numerous individuals with essentially normal health are referred by industrial, public health, or Army physicians to nephrologists for investigation of asymptomatic proteinuria or microscopic hematuria, or both. In most instances, these abnormalities are detected serendipitously by urinalysis during employment physical examinations or routine physical examinations, or following a visit to a physician for some kind of illness which is almost always unrelated to kidney or urinary conduits. By and large, these patients do not have any relevant past history that could contribute to the abnormal results of the urinalysis. Likewise, the physical examinations are essentially normal in these individuals.

Although abnormal results of a urinalysis in an otherwise healthy individual have no immediate serious import, a thorough investigation is deemed necessary in order to rule out a slowly progressive lesion, that is, focal glomerular sclerosis or another pathological process with a tendency to spontaneous remissions and exacerbations, e.g., membranous or mesangioproliferative glomerulonephritis (GN). It must be remembered, however, that it is difficult to convince patients of the necessity of renal biopsy study in the absence of symptoms. The physician must discuss with patients and their family members the potentially dangerous signals of an abnormal urinalysis and the value of renal biopsy study in the evaluation of these signals.

An editorial from the *British Medical Journal* (1973) has stated aptly that the term *focal glomerulonephritis* is applied indiscriminately to the clinical syndrome of recurrent hematuria. In two series of studies comprising 58 patients who were subjected to renal biopsy, a focal distribution was found in only one patient. The connotation of focal glomerulonephritis as a separate entity is questionable for two distinct reasons: (1) focal glomerular lesions can be observed in a variety of

conditions associated with diffuse glomerulonephritis, e.g., systemic lupus erythematosus (SLE), subacute bacterial endocarditis, Henoch–Schonlein syndrome; and (2) retrospective study of focal glomerulonephritis reveals that some or most of these cases have what is now believed to be focal glomerular sclerosis or sclerosing glomerulonephritis. (The causes of recurrent hematuria are listed in Table 11-1.)

Of many conditions in children, hereditary nephritis is being increasingly recognized as a common cause of recurrent microscopic hematuria (see details later); other common causes include subclinical poststreptococcal glomerulonephritis and mesangioproliferative GN. In the adult population, abnormal mesangium with and without deposits of immunoglobulins and focal glomerular sclerosis are frequently found in patients who present with microscopic hematuria or asymptomatic proteinuria or both.

PERSPECTIVES OF PROTEINURIA AND HEMATURIA

The abnormal results of a urinalysis should be considered from five different aspects, which are very similar to those stated by Dr. Clark West (1976): (1) proteinuria alone, (2) gross hematuria alone, (3) microscopic hematuria alone, (4) a combination of mild proteinuria and microscopic hematuria, and (5) any of the preceding with evidence of renal insufficiency. Either mild proteinuria alone or both mild proteinuria and hematuria almost always signifies renal parenchymal disease, whereas gross or microscopic hematuria alone does not necessarily do so (the term *proteinuria* referring to 24-hr protein excretion in excess of 200 mg, whereas *asymptomatic* or *mild proteinuria* refers to 24-hr protein excretion ranging from 0.2 g to 1 g or less). Because proteinuria alone and a combination of proteinuria and microscopic hematuria tend to have similar significance, they are discussed together. In the investigation of these subtle and nonspecific problems, certain rationales must be followed:

Table 11-1
Causes of Recurrent Hematuria

A. Diseases of the urinary conduits (ureter, bladder, urethra): stone, infection, combination of stone and infection, tumor
B. Diseases of the kidney
   1. Congenital: (a) polycystic disease, medullary cystic disease, solitary cysts; (b) angiomyolipoma associated with epiloia
   2. Acquired: (a) glomerular diseases, mesangiopathic GN, mesangioproliferative GN, membranous GN, focal glomerular sclerosis, mild diffuse proliferative GN, healed poststreptococcal GN; (b) tubulointerstitial diseases: chronic pyelonephritis, chronic graft rejection, carcinoma, infectious processes, e.g., tuberculosis, brucellosis, nephrocalcinosis; (c) undetermined—hereditary nephritis; (d) arterial diseases: idiopathic polyarteritis, allergic arteritis, severe hypertension, embolism from cardiac diseases, e.g., mitral stenosis, acute or subacute bacterial endocarditis; (e) venous diseases, chronic renal vein thrombosis

1. No major workup should be initiated on the basis of a trace to 1+ protein in the urine of an individual who has been on his feet or active prior to collection of the sample.

2. The presence of 4+ protein in a sample of urine, regardless of the time of collection and the position of the subject, should arouse suspicion of a renal parenchymal disease.

3. A small amount of protein (2+ to 3+) in the urine voided immediately after waking in the morning is suspicious of a renal parenchymal disease.

4. The patient should not be subjected to an invasive procedure, e.g., renal biopsy, without establishing proteinuria by quantitative estimation of protein in 24-hr urine on more than one occasion.

5. Conferences should be held with the patient and his or her family to discuss the potential dangers of persistently abnormal urinary findings and the values of renal biopsy studies. It should be stated that an undetermined but small percentage of patients with asymptomatic proteinuria, with or without hematuria, demonstrate renal parenchymal diseases which may progress and develop into renal insufficiency after an indefinite period. Only the renal biopsy study will allow the determination of the type of renal lesion by which the course of the disease can be ascertained.

Approaches to the Problem

1. A thorough physical examination is mandatory.

2. Laboratory tests. Except for 11 and 12, all the tests listed in Chapter 3 should be ordered. Of these, intravenous pyelography (IVP) is an ideal procedure for screening of tumor or cystic disease of the kidney, both of which may account for microscopic hematuria; it is adequate to rule out hydronephrosis, calculi, medullary cystic disease, and many congenital anomalies of the kidneys or urinary conduits. If IVP offers an equivocal diagnosis of any one of these conditions, further evaluation by retrograde pyelography or renal arteriography (bilateral or selective) must be recommended. Furthermore, if IVP is unequivocally normal, there is still a remote chance of finding anatomical abnormalities by retrograde pyelography or arteriography.

Other important tests include determinations of 24-hr urinary calcium and uric acid. Hypercalciuria and hyperuricosuria may suggest calcium and uric acid stones, respectively, both of which can cause hematuria.

3. Percutaneous renal biopsy. This invasive procedure should be undertaken under the following circumstances: (a) if 24-hr proteinuria is consistently greater than 0.5 g; (b) if there is negative evidence for tumor, cyst, and congenital abnormalities of the kidney, all of which may be the cause of microscopic or gross hematuria; (c) in the presence of both proteinuria and hematuria; or (d) if there is evidence of impaired renal function.

If renal biopsy is performed, the tissue must be fixed for light microscopy (LM), electron microscopy (EM), and immunoflourescence microscopy (IFM)

studies. The high yield of renal biopsy study in the investigation of hematuria syndrome is supported by the observations of several investigators. Some authors have contended that renal biopsy study, in particular using IFM, is important in order to rule out or confirm IgA nephropathy.

The value of renal biopsy study can be seen by the results now presented of the renal biopsy studies in our own series, and the brief account of the observations made by other investigators that follows.

Our series includes 29 patients who presented with microscopic hematuria alone, microscopic hematuria and mild proteinuria, or mild proteinuria alone. These patients were asymptomatic and the physical examinations were normal in all but three patients. Three patients had mild hypertension, and in no patient was the blood pressure higher than 160/100 mm Hg. The patients with azotemia were excluded from the analysis, nor does this analysis include patients in whom LM revealed definite abnormalities, e.g., diffuse proliferative, membranous, or mesangioproliferative changes or segmented and focal glomerular sclerosis. This analysis also excludes patients who had evidence of systemic disease, e.g., SLE, gammopathy, or metabolic diseases.

Renal tissues were studied by electron microscopy in all but two patients and by immunofluorescence microscopy in more than half of the patients. Light microscopy revealed normal morphology in most cases. Mild mesangial or segmental proliferative change and a slight increase in mesangial matrix were observed in a few cases.

It can be seen from the EM analysis in Table 11-2 that EM observations were normal or nondiagnostic in 10 patients (33%) and diagnostic in the remaining 19 paitents (66%). The diagnosis of healing poststreptococcal GN was made on the basis of a few typical "humps" without the presence of proliferative change or exudation of PMN. The immune complex GN was characterized by discrete deposits in the epithelial, subendothelial, or intramembranous locations. Electron microscopy study has helped the diagnosis of diffuse proliferative or extracapil-

Table 11-2
Analysis of Electron Microscopy Studies of Renal Biopsies from 29 Patients with Hematuria Alone, Hematuria and Proteinuria, and Proteinuria Alone

Categories of EM diagnoses	Hematuria alone (N = 25)	Hematuria and proteinuria (N = 3)	Proteinuria alone (N = 1)
Normal	5	0	0
Nondiagnostic	5	1	0
Mesangiopathic GN	5	0	0
Mild extracapillary proliferative GN	4	0	0
Mild diffuse proliferative GN (without deposits)	2	0	0
Early stage of hereditary nephritis	2	1	0
Immune complex GN	1	1	1
Poststreptococcal GN	1	0	0

Table 11-3
Results of Immunofluorescence Studies of Renal Biopsies[a]

Staining material	Hematuria only (13)	Hematuria and proteinuria (2)	Proetinuria only (1)
IgG	–	+++ (1)	++
IgM	+ (1), ++ (1)	–	-
IgA	+ (1), ++++ (1)	–	–
IgE	–	–	–
C3	+	+++ (1)	+
Fibrinogen	–	–	–
Albumin	–	–	–

[a] Symbols: –, negative; +, trace; ++, slight; +++, moderate; ++++, marked.

lary proliferative GN in 16% of the patients and mesangiopathic GN in 16% of the patients. The causative factor(s) for these pathological states could not be ascertained by EM studies.

Renal biopsies were studied in 16 patients by immunofluorescence microscopy (see Table 11-3). The IFM was ambiguous or inconclusive in the vast majority of the biopsies studied. A heavy deposit of IgA was found exclusively in one biopsy. With the exception of occasional patients, the IFM study in our series failed to support immunological mechanisms in the pathogenesis of renal diseases associated with hematuria, mild proteinuria, or both. We will look at the follow-up of some of these patients, which, along with the follow-up studies in other series, might help in understanding the natural history of this syndrome. However, we will first concentrate on analyzing the undefined entity called mesangiopathic or mesangial GN.

MESANGIOPATHIC GLOMERULONEPHRITIS

SYNONYMS. Berger's disease, immunoglobulin A glomerulonephritis, focal glomerulonephritis.

This is a morphological abnormality in the kidney which has not yet been delineated because of (1) lack of knowledge of etiological factor(s), (2) unclear pathogenetic mechanism, and (3) lack of long-term follow-up of initial clinical and histological changes in a large number of patients.

Pathology

The histopathological studies in one of our patients are illustrated here to exemplify Berger's disease.

Patient #1. S.Mc., an 18-year-old white male, was found to have microscopic hematuria during a routine physical examination. His physical examination, including blood pressure, was completely normal, as were the laboratory studies. A percutaneous renal

biopsy was done and the renal tissue was studied by LM, EM, and IFM. Light microscopy was nondiagnostic (Figs. 11-1 and 11-2). A diagnosis of mesangiopathic GN was made by EM, which demonstrated conspicuous mesangial changes characterized by excessive basement membrane (BM)-like materials, an increased number of mesangial cells, and small amounts of electron-dense deposits (Figs. 11-3 and 11-4). The electron-dense deposits were located within the mesangium and in the centrilobular portions of the capillaries (Figs. 11-3 and 11-4). The peripheral capillary loops were essentially normal (Fig. 11-3). The other component parts of the kidney such as tubules and interstitium appeared normal. IFM study revealed massive deposition of IgA, predominantly in the mesangium, with little or none in the peripheral capillary loops (Fig. 11-5). There was slight or no deposition of other immunoglobulins or C3. Follow-up studies 3 years after the initial visit were unchanged.

Diagnosis

The French investigators have indicated that the diagnosis of Berger's disease can be made essentially by IFM study showing mesangial deposition of IgA.

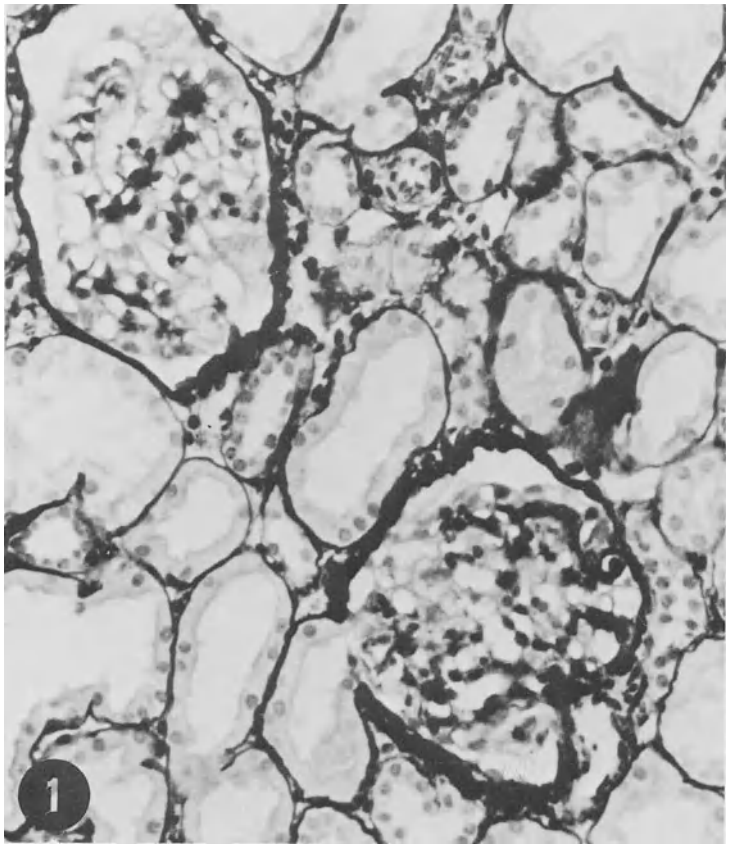

Fig. 11-1. Except for slightly prominent mesangium the glomeruli appear normal. Tubules are intact (PAMS, ×120).

Questions arise as to how specific and dependable this IFM finding is in conferring a pathological diagnosis and how this finding explains the clinical syndrome. Levy and colleagues (1973) from France and Vernier and associates (1975) from the United States have stated that deposition of IgA–IgG in the mesangium is not unique in patients with recurrent hematuria, since a similar pattern can be

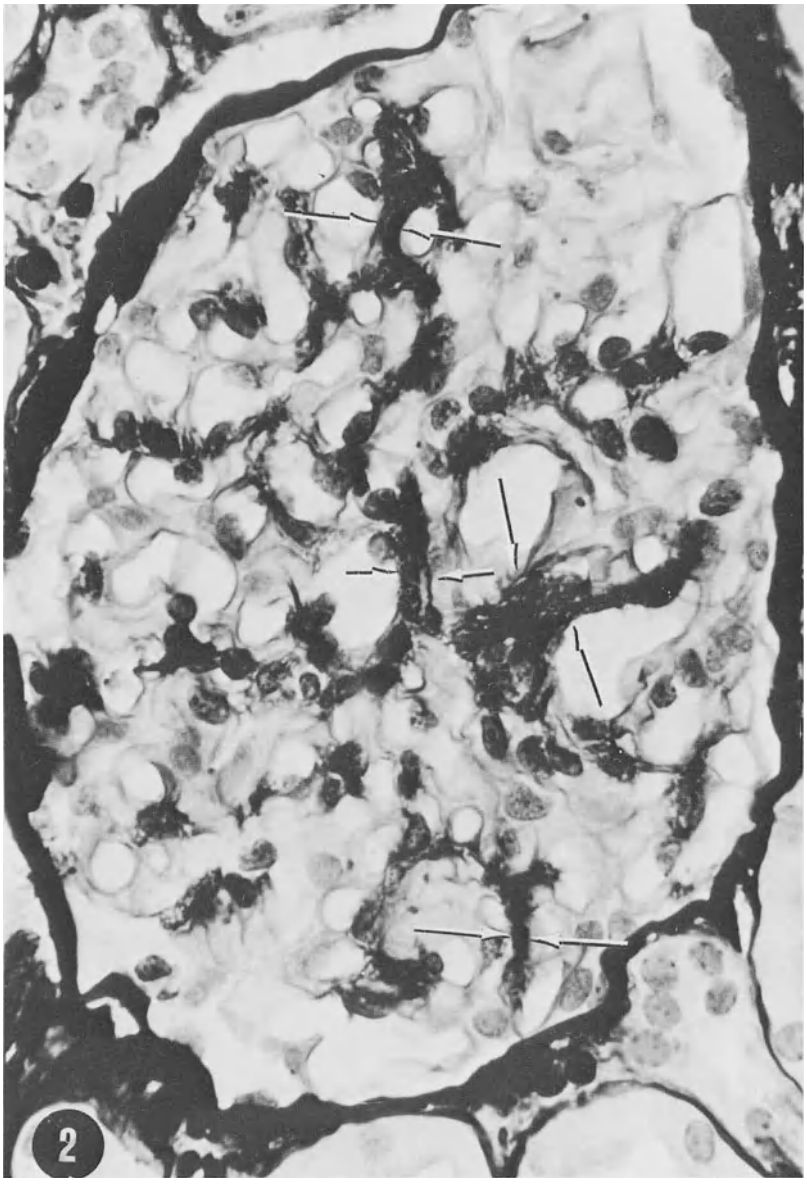

Fig. 11-2. This glomerulus reveals conspicuous mesangium (opposing arrows), the conspicuousness being mainly due to excessive silver-positive matrix. The peripheral capillary loops are essentially normal (PAMS, ×320).

observed frequently in GN of anaphylactoid purpura (Henoch–Schonlein syndrome) and rarely in lupus nephritis. These authors, however, have conceded that regular distribution of IgA–IgG in all glomeruli, especially when they appear normal by LM, makes it meaningful to consider this deposition as a participant in the pathogenesis of recurrent hematuria syndrome. The French investigators also suspect Berger's disease on the basis of electron-dense deposit found solely in the mesangium.

The IFM finding of the presence of C3 along with other immunoglobulins, supplemented by electron-dense deposits in the mesangium, provides a solid ground upon which to accept this process as one type of glomerulonephritis. Finlayson and co-workers (1975) believe that IgA glomerulonephritis is a distinct

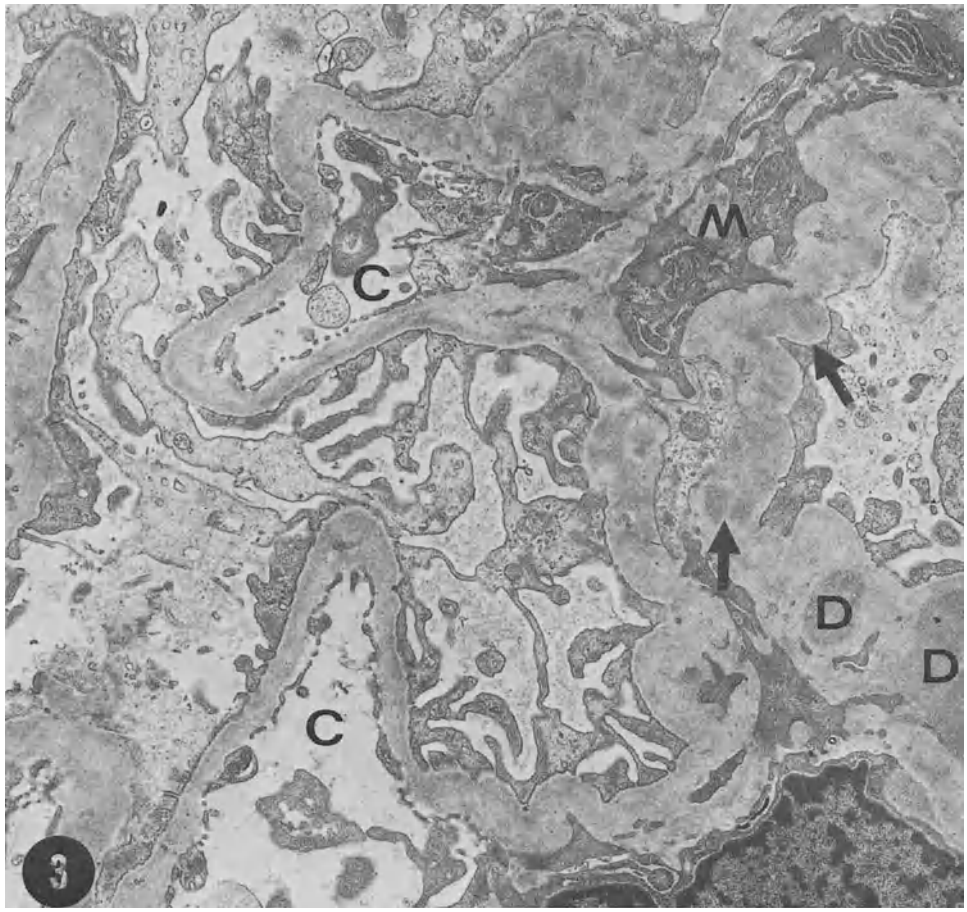

Fig. 11-3. Note conspicuous deposits (D and arrows) in the centrilobular portions of a capillary loop. The peripheral capillary loops (C) appear essentially normal. Thickened mesangium (M) is shown (UA + LC, ×7000).

immunological entity and is characterized by deposition of large amounts of IgA predominantly within the mesangium and by histopathological changes (LM and EM) mostly in the mesangium.

The serious obstacle to recognizing Berger's disease is an analogous clinicopathological syndrome described by Van de Putte *et al.* (1974). These authors performed renal biopsies on 47 patients with recurrent microscopic or persistent microscopic hematuria, unexplained by urinary tract abnormalities or manifest renal disease. Histological examination revealed mesangial proliferative GN in 34 patients. Granular deposits of immunoglobulins and complement components

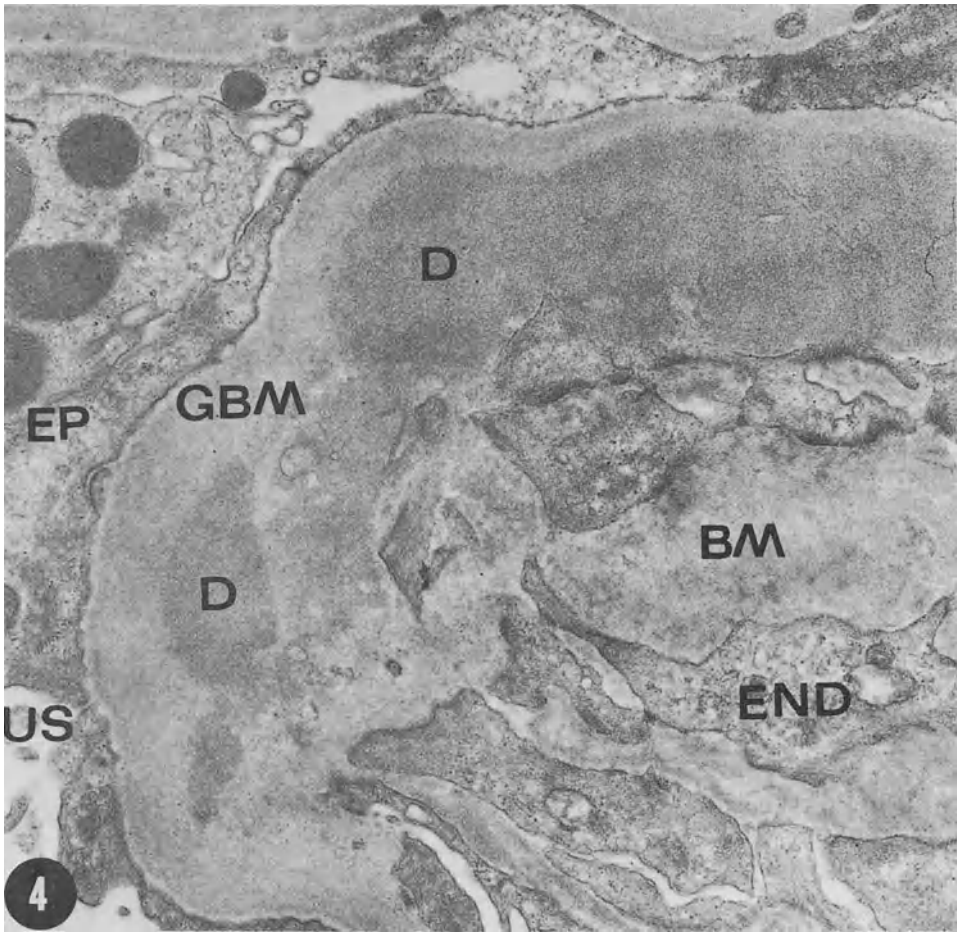

Fig. 11-4. A magnified view of the centrilobular (mesangial) portion of a glomerular capillary demonstrates subendothelial discrete electron-dense deposits (D) and excessive basement membrane-like materials (BM) obscuring most of the lumen and endothelial cell (END) of the capillary. Glomerular basement membrane (GBM) appears essentially normal. Epithelial cell (EP) and urinary space (US) are shown (UA + LC, ×21,000).

were found in the glomerular mesangium in 15 of 24 biopsies studied by IFM; IgM, BIC, and Clq in all; IgA in ten; and IgG in only two. The intensity of immunofluorescence staining correlated with the glomerular proliferative reaction as judged by light microscopy. Electron-dense granular deposits in the mesangial area were found exclusively in patients with active disease. Thus, the authors concluded that glomerulonephritis in these patients was caused by IgM and IgA antibody-associated mesangial immune complex deposition with activation of the classic complement pathway.

Pathogenesis of Mesangiopathic Glomerulonephritis (Berger's Disease)

It is fairly well established that the mesangial cell acts as a scavenger. It has been shown that injected macromolecules such as heat-aggregated IgG are readily taken up by the mesangium of normal rats and that greatly increased quantities enter the mesangium in experimental nephrotic syndrome and nephrotoxic serum

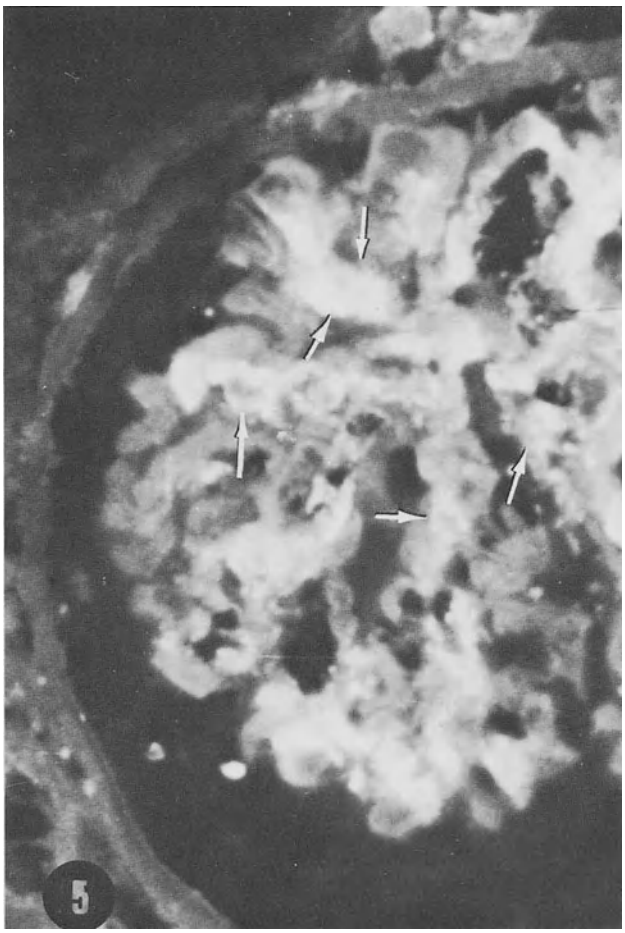

Fig. 11-5. This glomerulus shows heavy deposits of IgA in the mesangium (arrows) with little or none in the peripheral capillary loops. The fluorescence for IgG, IgM, IgE, fibrinogen, and C3 was negative (IFM, ×1600). The micrograph was kindly provided by Dr. Samuel R. Oleinick, Immunology Section, VA Hospital, Oklahoma City, Oklahoma.

nephritis. Applying this analogy, it may be postulated that bacterial or viral antigens from episodes of upper respiratory infections which often accompany hematuria syndrome may accumulate in the mesangium. These trapped antigens may incite the production of antibodies, which then bind the antigens and form immune complexes within the mesangium. This is purely hypothetical but seems appropriate. The hypothetical issue should stimulate investigation to confirm the possibility that this mesangial change represents the initial stage of some diffuse process, e.g., mesangioproliferative GN or sclerosing GN. Recurrence of the disease process with predominant deposition of IgA in the mesangium of a transplanted kidney supports the idea of a distinct disease entity.

The relationship between elevated serum IgA levels and the mesangial deposition of IgA is unclear. An interrelationship is conceivable if reduction of serum IgA level rather than elevation is found. This would then be analogous to dense deposit disease in which massive deposition of C3 in the glomeruli is associated with markedly low serum C3. Elevated serum IgA levels may mean abnormal immune mechanism of the IgA class, but they do not explain the affinity of IgA for the glomerular mesangium and the elevation in the serum despite its heavy deposition in the kidney.

Clinical Profiles and Course of IgA Glomerulonephritis (Berger's Disease)

Some highlights of the clinical profiles reported by Levy *et al.* (1973), Finlayson *et al.* (1975), and Van de Putte *et al.* (1974) are included here. Levy and colleagues studied 33 children, of which 30 had recurrent gross hematuria and three had recurrent microscopic hematuria. The notable features reported are as follows:

1. The disease was more common in boys than girls.
2. It was more common after 3 years of age (50% of the children were between 6 and 12 years).
3. Almost 80% of the children had no preceding history. About 20% of the children admitted to repeated infections of the upper respiratory tract.
4. The episodes of hematuria seemed to coincide with illnesses involving an IgA-secreting organ system, such as respiratory tract or intestinal tract.
5. The intervals between the episodes varied from 10 days to 2 years with an average of several months. The intervals were shorter in the early stage of the syndrome.
6. The duration of single episodes of hematuria rarely exceeded 15 days, being no more than 3 days in 60% of the children.
7. Infection by hemolytic streptococci was proven in less than 10% of the children. The interval between infectious episodes and gross hematuria generally was less than 2 days, hematuria sometimes appearing on the same day as the throat symptoms.
8. There was variable proteinuria but seldom was it greater than 1 g in a 24

hr period. Microscopic hematuria persisted between episodes of gross hematuria in about 20% of the children.

9. Serum C3 was always normal.

10. Elevation of serum IgA levels was a frequent but not a consistent finding. For example, Finlayson *et al.* found significant elevation of serum IgA in eight patients tested, compared to controls (4.3 ± 1.68 versus 2.25 ± 0.85 mg/

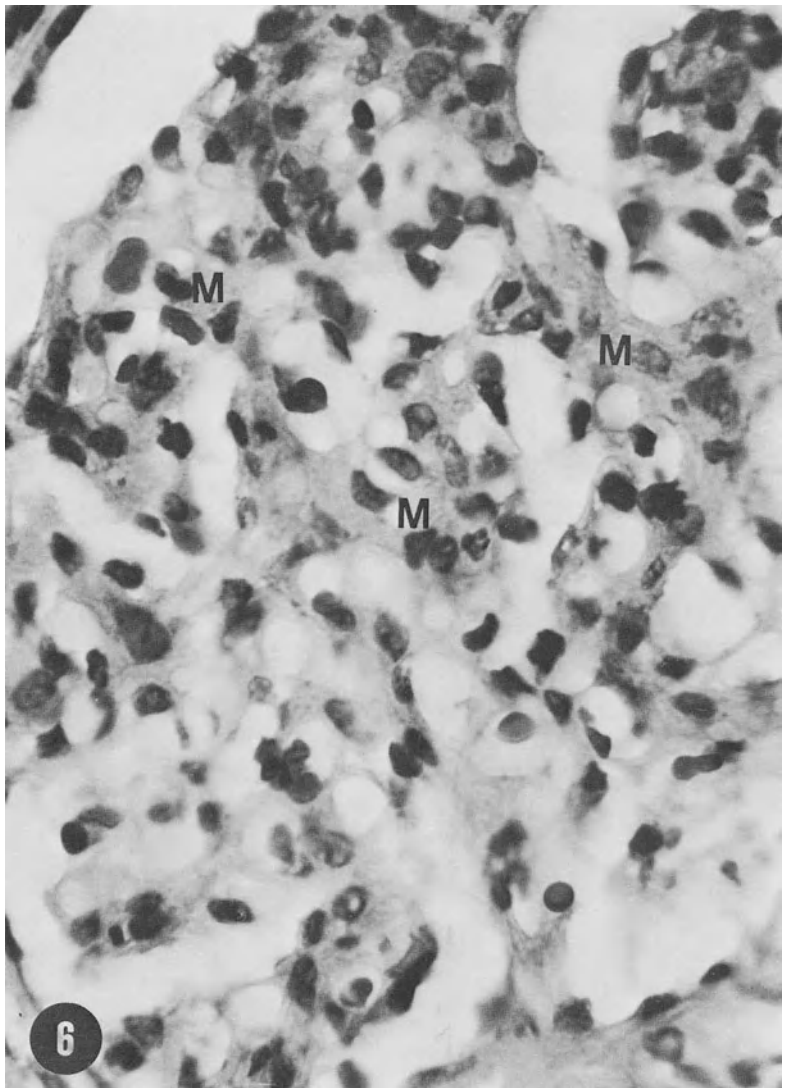

Fig. 11-6. The upper half of this glomerulus shows mild hypercellularity and slight increase of matrix in the mesangium (M) which demarcates the upper half from the lower half. The lower half of the glomerulus appears essentially normal (H & E, ×320).

ml; $p < 0.05$). They also found significant elevation of nasal IgA concentration in five patients tested, compared to controls (0.52 ± 0.47 versus 0.18 ± 0.08 mg/ml; $p < 0.05$).

11. Follow-up from 1 to 15 years demonstrated no renal insufficiency in any patient. Proteinuria of 0.5 to 1 g persisted for several months after the last episode of hematuria in six children.

Other investigators have reported renal failure in about 10% of their patients,

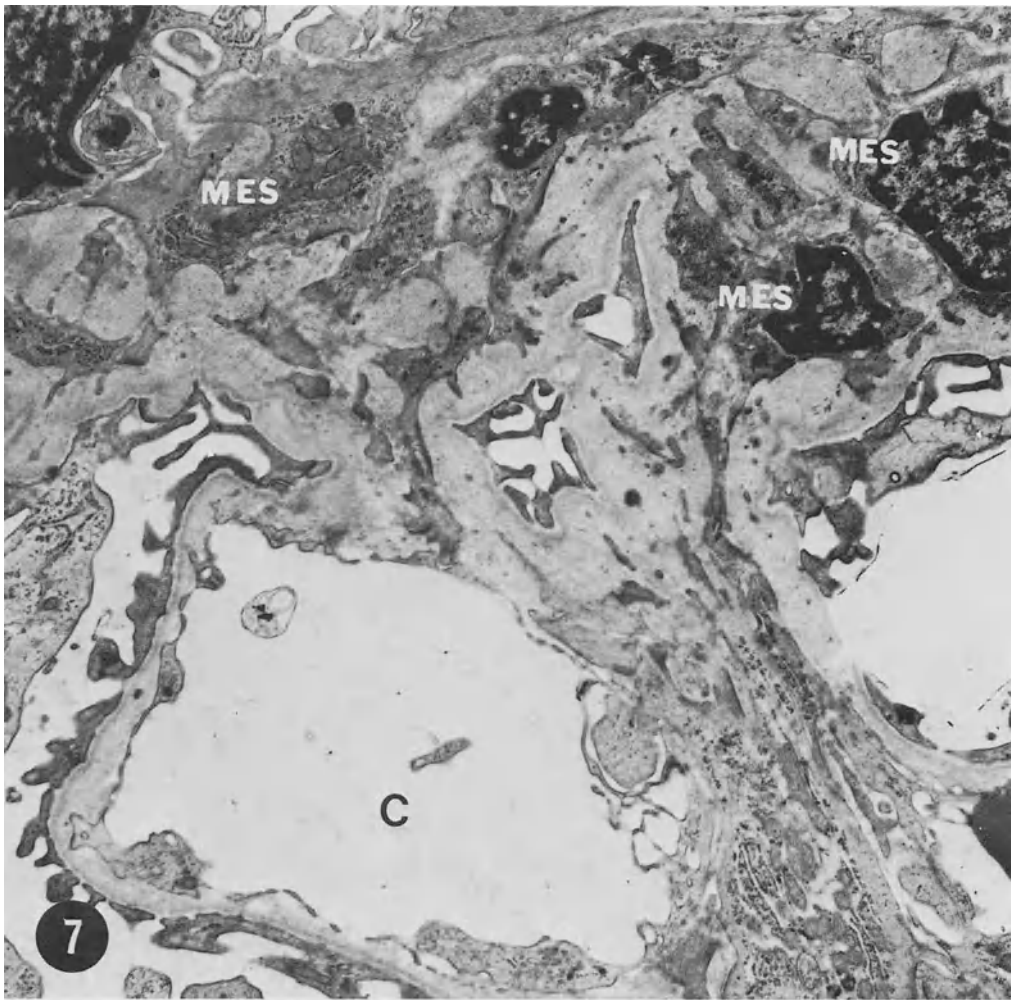

Fig. 11-7. The presence of many mesangial cells (MES) and a slight increase of mesangial matrix in the glomerulus (shown in Fig. 11-6) are confirmed by EM. The peripheral capillary loop (C) appears normal (UA + LC, ×8000).

more in adults than children. By serial renal biopsy studies, Van de Putte and co-workers observed no progression of the initial renal lesions or appearance of abnormal renal histology in patients whose renal biopsies were initially interpreted as normal.

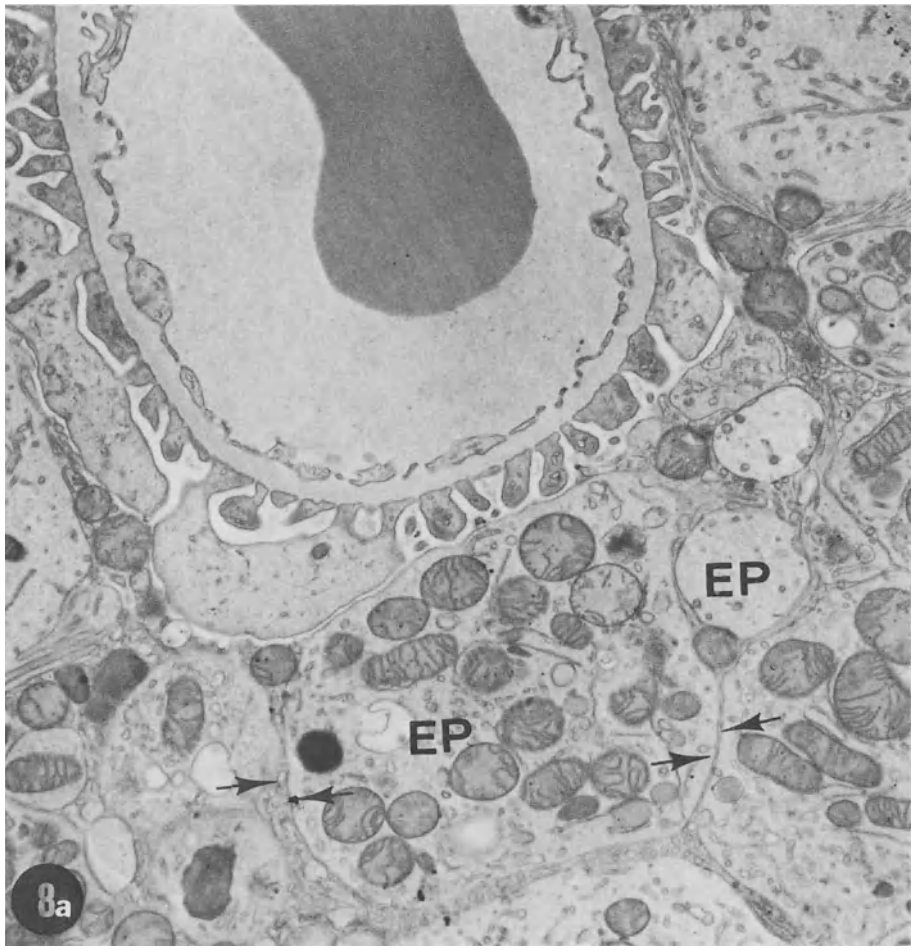

Fig. 11-8. (a) An excessive number of epithelial cells (EP) surround a glomerular capillary. These epithelial cells appear to be proliferative and active because of the presence of many mitochondria. Adhesions between EP are shown by opposing arrows (these epithelial cells are unlikely to be herniated tubular epithelial cells). The glomerular capillary has no demonstrable abnormality (UA + LC, ×15,000). (b) This micrograph reveals glomerular epithelial changes almost identical to those shown in (a). A distal convoluted tubule (DCT) is seen. The cortical interstitium (I) is narrow and contains some collagen fibers. Visceral epithelial cells (VEP) and adhesion (opposing arrows) between VEP and parietal epithelial cells (PEP) are shown (UA + LC, ×2500). From the renal biopsy of an 8-year-old male child who was referred to West Suburban Hospital, Renal Division for investigation of microscopic hematuria. His physical examination was normal as were the laboratory studies. (As a part of author's study during research fellowship with Dr. Robert C. Muehrcke, West Suburban Hospital, Oak Park, Illinois.)

A patient is presented here to demonstrate the benign nature of the disease process.

Patient #2. C.F., a 55-year-old white male, was admitted to the Oklahoma City VA Hospital in October 1972 for investigation of microscopic hematuria, which was found during a routine physical examination. Physical examination was normal except for mildly elevated blood pressure of 162/98 mm Hg. The routine laboratory studies were normal, including normal serum urea nitrogen and creatinine concentrations, which were 16 and 1.2 mg/100 ml, respectively. The urological investigations, including intravenous pyelogram, cystoscopy, and retrograde pyelogram, revealed normal findings. A percutaneous renal biopsy was done and the renal tissue was studied by LM and EM. The LM showed two hypercellular segments in one glomerulus and slightly wide and hypercellular mesangium in other glomeruli (Fig. 11-6). EM demonstrated wide mesangium with excessive BM-like materials but no deposits. (Fig. 11-7). Follow-up studies 4 years after the initial visit showed persistence of microhematuria, and no change in blood pressure and renal function.

Comments. The histopathological study suggests mesangiopathic glomer-

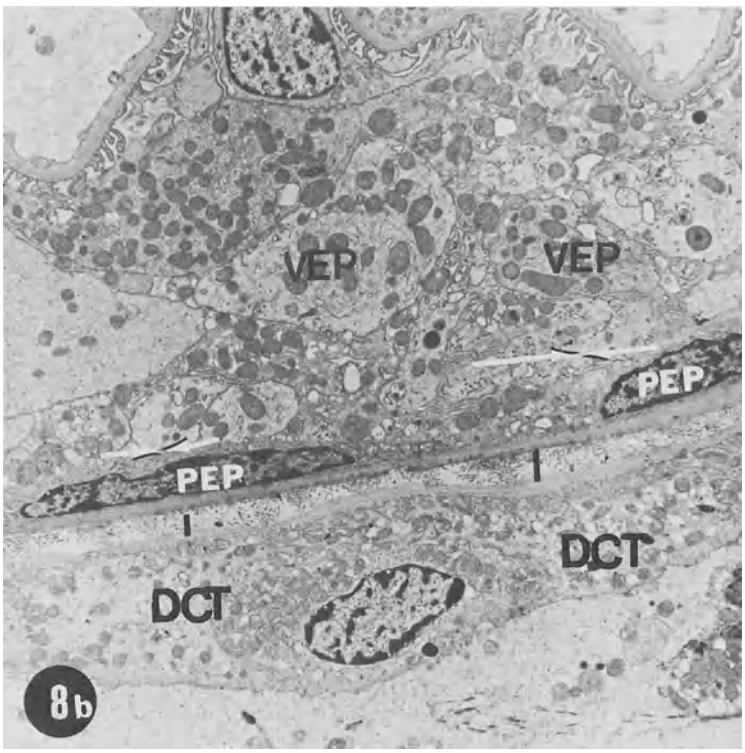

Fig. 11-8. *(Continued)*

ulonephritis in this patient. Since IFM was not done, it was difficult to support a diagnosis of Berger's disease according to definition.

OTHER TYPES OF RENAL PATHOLOGY, CLINICAL PROFILES, AND COURSE OF THE HEMATURIA AND ASYMPTOMATIC PROTEINURIA SYNDROME IN OUR SERIES

Example of Mild Extracapillary Proliferative GN

Patient #3. R.G., a 13-year-old white male, was admitted to Oklahoma Children's Memorial Hospital in November 1972 with a 4-day history of chocolate-colored urine.

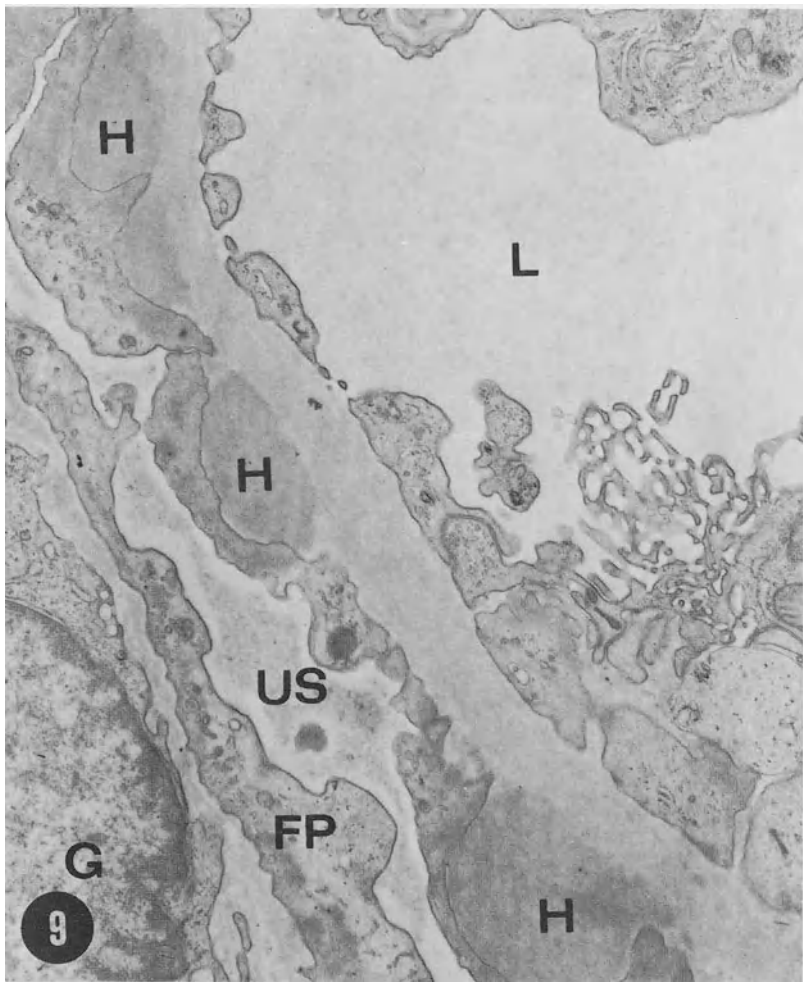

Fig. 11-9. A glomerular capillary reveals several typical "humps" (H). No cellular proliferation or inflammatory exudation is seen. Lumen of the glomerular capillary (L) is shown. A portion of another glomerular capillary (G) shows fusion of foot processes (FP). The urinary space (US) between the two capillaries can be seen (UA + LC, ×22,000).

There was no identifiable illness preceding the appearance of this abnormal urine. Past history revealed an episode of hematuria 9 years earlier, whereupon a renal biopsy was performed and reported to be normal. The physical examination had always been normal. A urinalysis revealed 50 to 100 RBC/HPF, and no protein or casts. The 24-hr urinary protein was less than 5 mg/dl. He had a serum urea nitrogen of 7 mg/100 ml, ASO titer of 333 Todd units, and serum C3 of 100 mg/100 ml. A second renal biopsy was performed and the renal tissue was studied by LM and EM. LM study revealed normal morphology; EM showed exclusive proliferation of visceral and parietal epithelial cells and adhesions between the epithelial cells (see Figs. 11-8a and 11-8b). A follow-up study 3 years after the second renal biopsy revealed the presence of normal urinalysis, blood pressure, and renal function tests.

Example of Poststreptococcal GN

Patient #4. R.B., an 11-year-old black male, was admitted to Oklahoma Children's Memorial Hospital in December 1972 with a history of hematuria for 1 month, and malaise and vomiting for 3 to 5 days prior to admission. Physical examination including blood

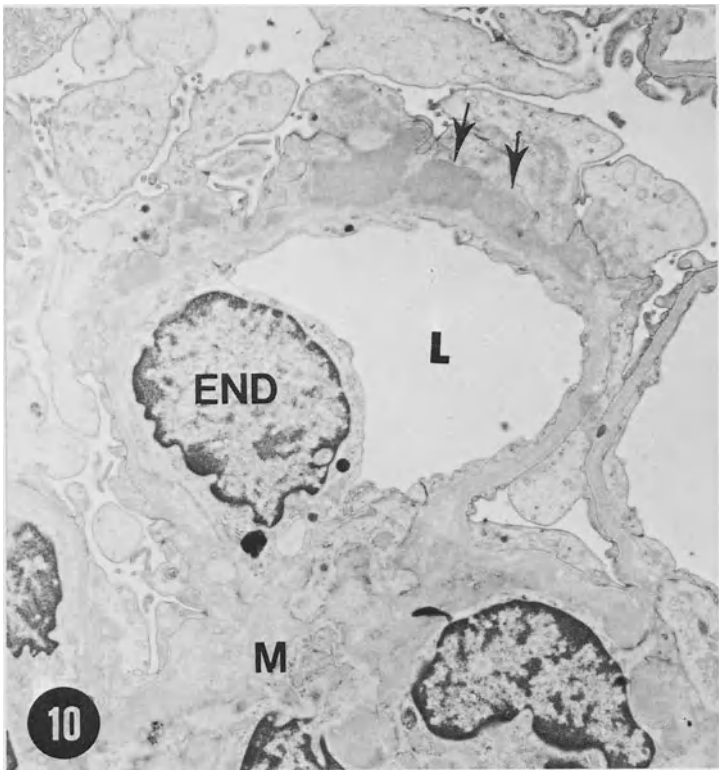

Fig. 11-10. This glomerular capillary demonstrates several electron-dense deposits (arrows) on the epithelial aspect. These deposits may be described as atypical "humps." Lumen of the capillary (L), endothelial cell (END), and mesangium (M) are shown (UA + LC, ×4000). (As a part of author's study during research fellowship with Dr. Robert C. Muehrcke, West Suburban Hospital, Oak Park, Illinois.)

pressure was normal. Laboratory investigations—Urinalysis: protein 4+, RBC too numerous to count, some RBC casts (a repeat urinalysis a few days later showed 10 to 15 RBC/HPF and no RBC casts); serum chemistry: urea nitrogen and creatinine 11 mg/100 ml and 0.6 mg/100 ml, respectively; throat culture normal flora; ASO titer normal; serum C3 155 mg/100 ml; 24-hr urinary protein 380 mg; 24-hr endogenous creatinine clearance 81 ml/min. A percutaneous renal biopsy was done and the renal tissue was studied by LM, EM, and IFM. LM revealed mild proliferative glomerulonephritis. EM demonstrated several typical "humps" in a few glomerular capillaries. There was no proliferative or exudative change observed (Fig. 11-9). IFM study showed lumpy (granular) deposits of IgG and β_{1c} (C3) globulin in the peripheral capillary loops. The patient was lost to follow-up.

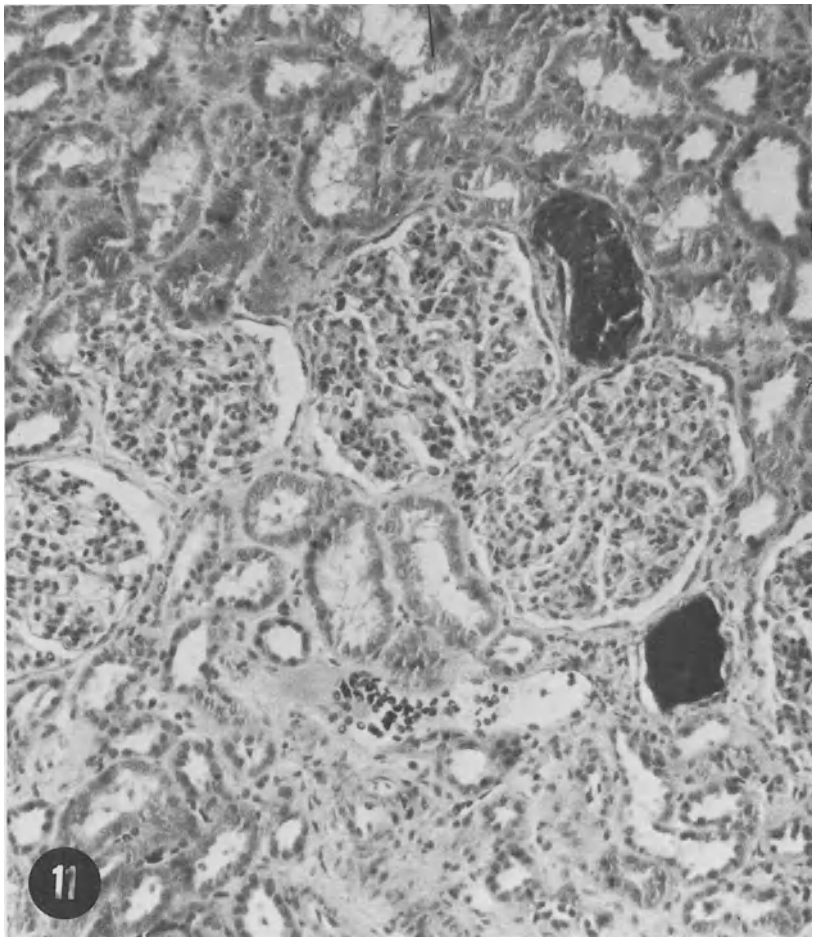

Fig. 11-11. Several glomeruli reveal normal to slightly hypercellular appearance. Two dilated tubules are filled with blood. An island of normal cortical tissues is seen in the upper half of the figure. Slight interstitial fibrosis is clearly observed in the lower half (H & E, ×80). From the renal biopsy of B.B., a 3-year-old Indian female who was found to have microscopic hematuria and 2+ protein in urine in a preschool physical examination. The most significant history was that her 11-year-old brother had deafness and severe hypertension in addition to microscopic hematuria and proteinuria. A diagnosis of hereditary nephritis was made in the brother.

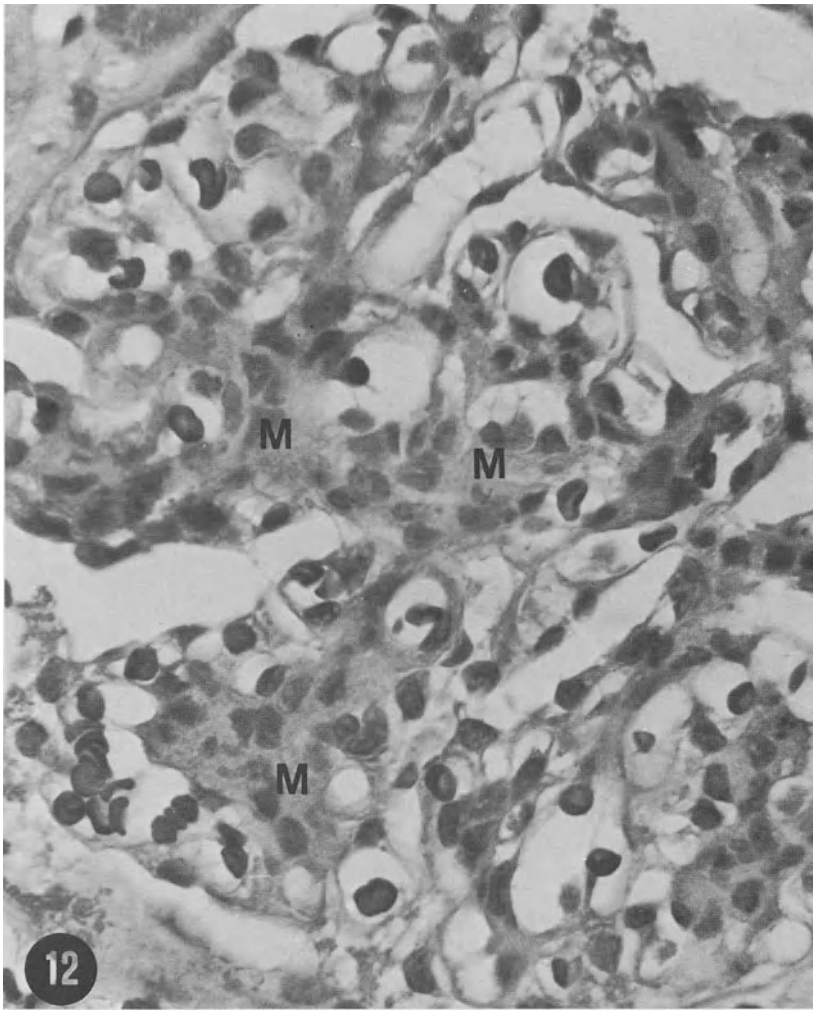

Fig. 11-12. In this glomerulus increase of cellularity and matrix in the mesangium (M) is noted (H & E, ×320). From the same patient as Fig. 11-11.

Comments. EM and IFM studies indicated poststreptococcal glomerulonephritis of 3 to 4 weeks' duration. This histopathological diagnosis was not supported, however, by the negative history of sore throat, normal ASO titer, and normal serum C3 concentration (see also patient #7, E.G.. in Chapter 12).

Example of Immune Complex Glomerulonephritis*

Patient #5. V. S., a 3-year-old white female, was admitted to West Suburban Hospital, Oak Park, Illinois, in February 1967 for investigation of repeated episodes of

* This was done as part of the author's study during a research fellowship with Dr. Robert C. Muehrcke, at West Suburban Hospital, Oak Park, Illinois.

dark urine. Some of these episodes were accompanied by abdominal cramps and fever. Although sore throat, upper respiratory tract infection, and skin rashes were denied, hematuria cleared each time following administration of penicillin or sulfonamides. Hematuria recurred almost always following withdrawal of antibiotic or sulfa drugs. Laboratory studies revealed essentially normal values. These included normal ASO titer and serum C3 level. The 24-hr urinary protein was 270 mg. A percutaneous renal biopsy was performed and the renal tissue was studied by LM and EM. The LM study revealed mildly hypercelular glomeruli with slight or no exudation of PMN. EM study showed atypical "humps" but no hypercellularity or exudation (Fig. 11-10). Follow-up studies were performed every year for 5 years, and revealed normal physical examination and laboratory tests within normal limits.

NONDIAGNOSTIC ULTRASTRUCTURAL STUDIES

Nondiagnostic studies are those in which electron microscopy revealed changes that did not signify any specific diagnosis. These changes comprised

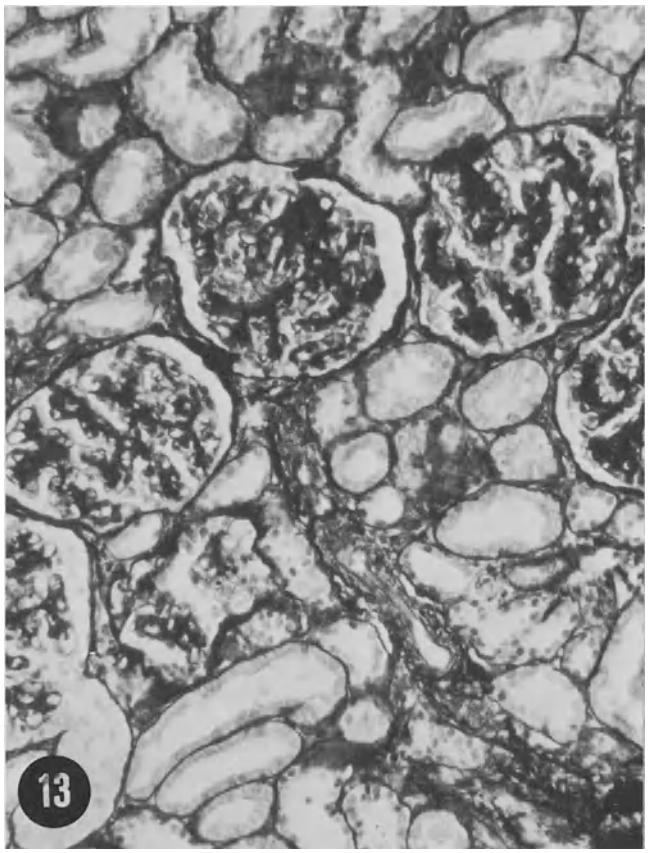

Fig. 11-13. Increase in mesangial matrix is clearly discerned in the glomeruli (PAMS, ×80). From the same patient as Fig. 11-11.

segmental fusion of foot processes in some of the glomerular capillaries, slight irregularities on the endothelial aspect of peripheral capillary loops, and slight increase in mesangial matrix. The LM morphology was essentially normal, and IFM studies did not show positive fluorescence for any of the staining materials. Follow-up clinical and laboratory studies in several patients with nondiagnostic renal pathology for periods of 12 to 36 months showed the presence or absence of microhematuria, but no proteinuria and normal renal function tests.

EARLY STAGE OF HEREDITARY NEPHRITIS

EM has helped significantly in establishing a diagnosis of hereditary nephritis at an early stage when microscopic hematuria is the only clinically detectable

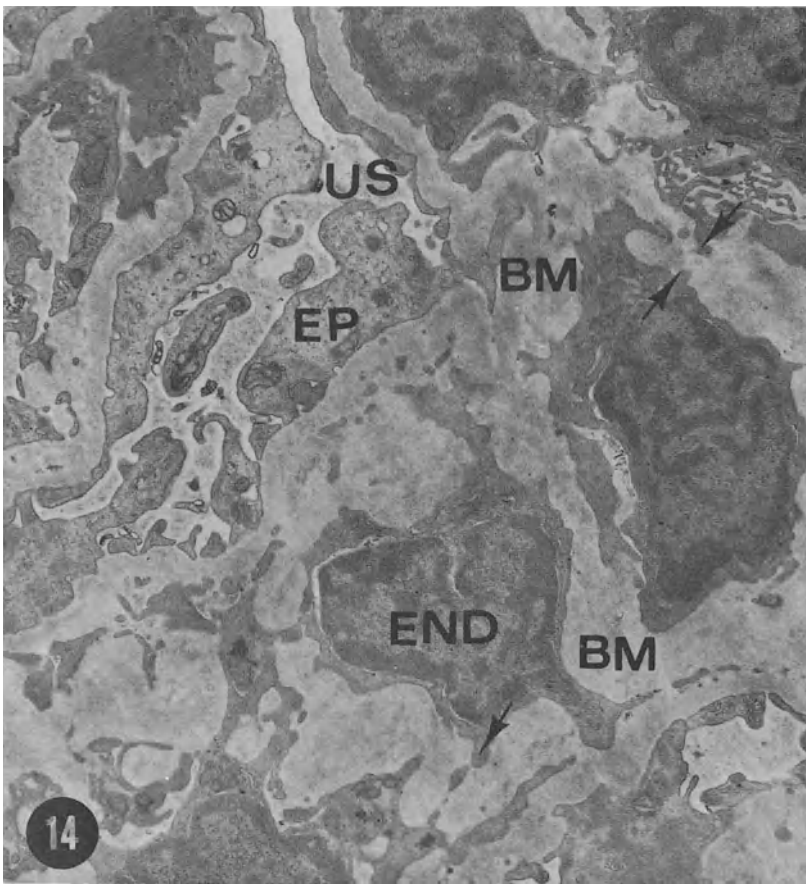

Fig. 11-14. Fragmentation of the glomerular basement membranes (opposing arrows), excessive basement membrane-like materials (BM), and atrophy of epithelial and endothelial cells (EP and END, respectively) are shown. Urinary space (US) is seen (UA + LC, ×15,000). From the same patient as Fig. 11-11.

abnormality. LM morphology at an early stage of this disease is either normal or shows mild increase in mesangial cellularity and matrix or mild segmental hypercellularity (Figs. 11-11 to 11-13).

EM has exhibited findings which have contrasted greatly with those observed by LM. These were characterized by irregular thickening of the GBM on the endothelial aspect, excessive BM-like material, especially in the mesangium, fragmentations of the GBM, linear electron density along GBM, granules or particles within GBM, and partial to complete atrophy of endothelial, epithelial, and mesangial cells (Figs. 11-14 to 11-17). Tubules demonstrated atrophic changes

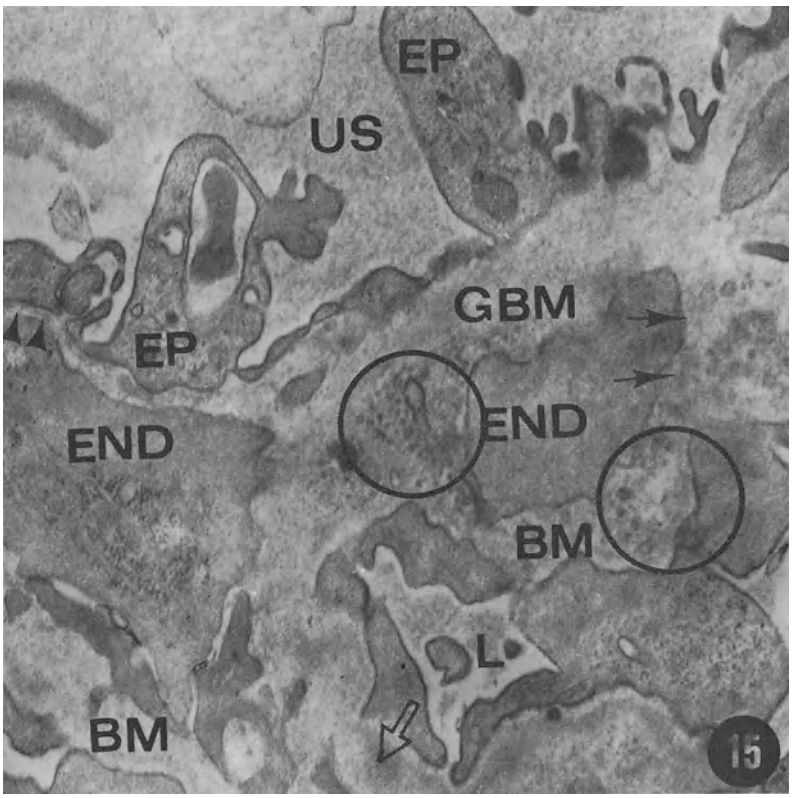

Fig. 11-15. This figure shows disruptive changes in the glomerular basement membrane (GBM) characterized by thickening (double arrows) and thinning (arrowheads) of GBM, basement membrane-like materials (BM) across the lumina (L) of glomerular capillaries with atrophy of endothelial (END) and epithelial (EP) cells. The collection of particles within the GBM (circles) is a conspicuous feature. Small electron-dense deposit (single arrow), and urinary space (US) are shown (UA + LC, ×21,000). From the renal biopsy of J.K., a 5-year-old white male who was referred to Oklahoma Children's Memorial Hospital. He gave a 3-months' history of dark-colored urine. The family history was significant: a maternal uncle was undergoing chronic hemodialysis, the mother had renal stones and repeated episodes of urinary tract infections, and a maternal cousin died at age 3 from renal disease.

and there were excessive amounts of collagen fibers in the interstitium (Fig. 11-18). We, as others, have not observed a marked discrepancy between light and electron microscopy findings in the study of glomeruli in conditions other than hereditary nephritis and, of course, lipoid nephrosis. It should be stated, however, that these EM findings cannot be considered specific for hereditary nephritis,

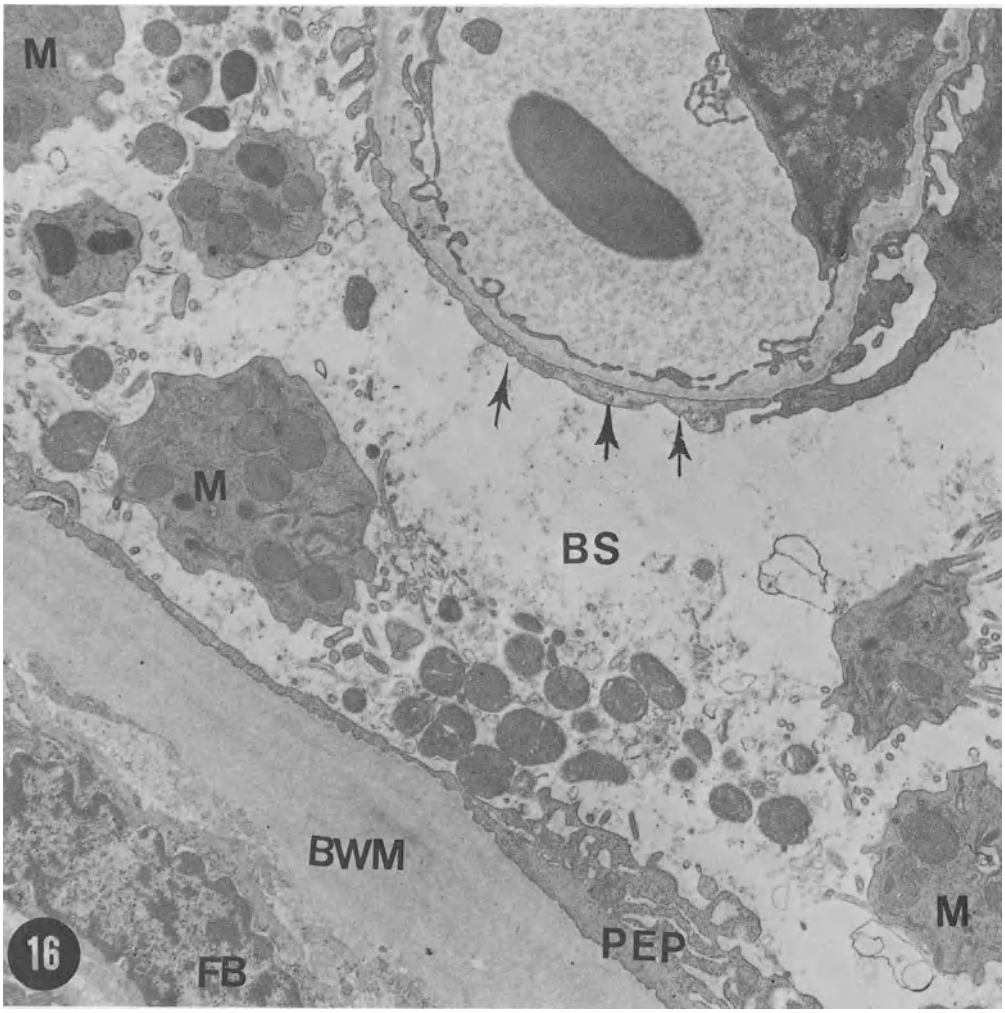

Fig. 11-16. This figure shows irregular thickening and thinning on the endothelial aspect of basement membrane, and segmental fusion of foot processes (arrows) in a glomerular capillary. The Bowman's space (BS) is quite conspicuous due to the presence of many macrophagelike cells (M) and many free mitochondria. Thickened Bowman's membrane (BWM), atrophic parietal epithelium (PEP), and a large fibroblast (FB) in the interstitium are seen (UA + LC, ×10,000). From the renal biopsy of K.K., a 9-year-old white female who was referred to Oklahoma Children's Memorial Hospital for investigation of persistent microscopic hematuria. Mother and mother's brother and sister were known to have hematuria. Her urinalysis revealed 8 to 10 RBC/HPF, 24-hr urine protein of less than 5 mg/100 ml, and normal serum urea nitrogen.

because such glomerular changes can be observed by EM alone in other types of glomerular diseases, e.g., anti-GBM type of glomerulonephritis and GN in Henoch–Schonlein syndrome. It has been emphasized by Hinglais and colleagues (1972) that the characteristic GBM alterations enumerated herein are not a consistent abnormality in hereditary nephritis. These authors found normal EM findings in seven cases of hereditary nephritis with a more benign course. They have studied the serial biopsies from some of their patients and observed progression of the pathological changes. Churg and Sherman (1973), however, have suggested that these glomerular lesions are specific for certain forms of hereditary nephritis.

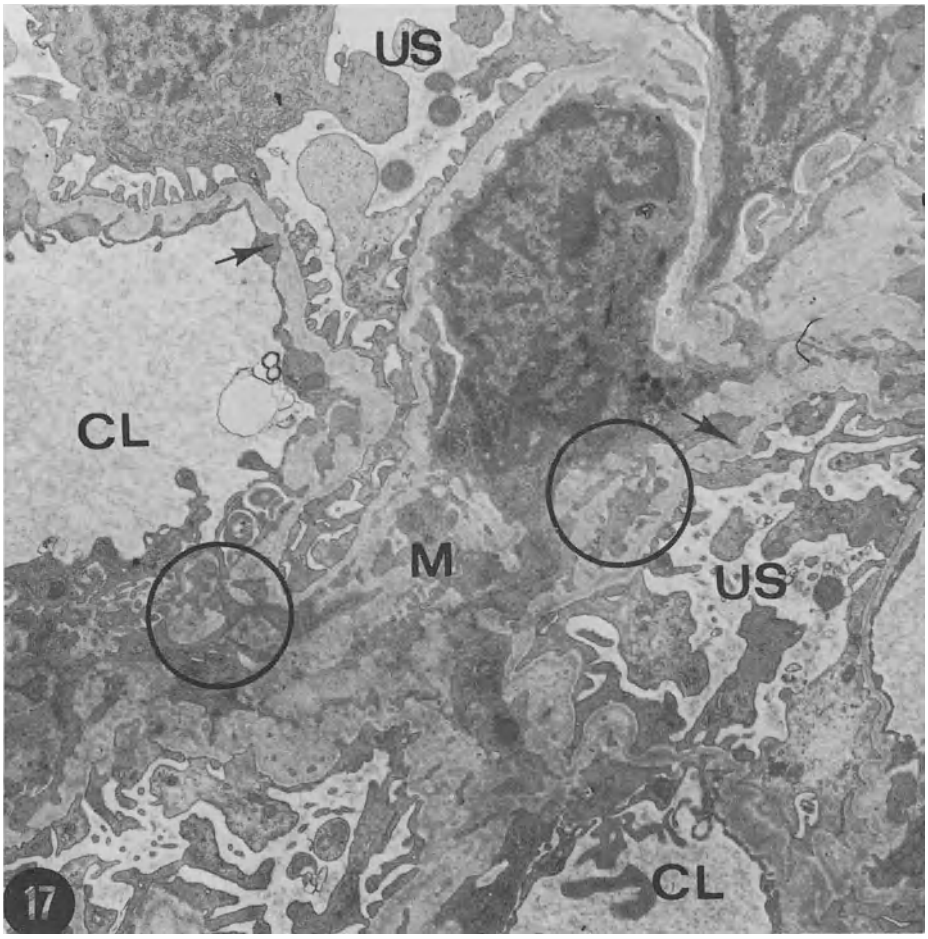

Fig. 11-17. In this electron micrograph, portions of many peripheral capillary loops and a mesangium are shown. Fragmentation of peripheral capillary basement membranes (arrows), small segments of basement membrane-like materials (circles), and a wide mesangium (M) constitute prominent features in this study. The lumen of the glomerular capillaries (CL) and urinary space (US) are shown (UA + LC, ×12,000). From the renal biopsy of the same patient as Fig. 11-16.

Interstitial foam cells were once considered a pathognomonic feature of hereditary nephritis. This is denied now by almost everyone because of improvement of our knowledge by extensive EM studies. However, I would like to state from my own experience with EM study that tubular and interstitial foam cells in the absence of hyperlipidemia strongly suggest hereditary nephritis. Since striking glomerular changes have been observed by EM in asymptomatic family members of patients with documented hereditary nephritis (Fig. 11-14), it may be argued retrospectively that EM study of renal biopsy in an asymptomatic stage may serve as a useful tool to predict the appearance of manifest hereditary nephritis.

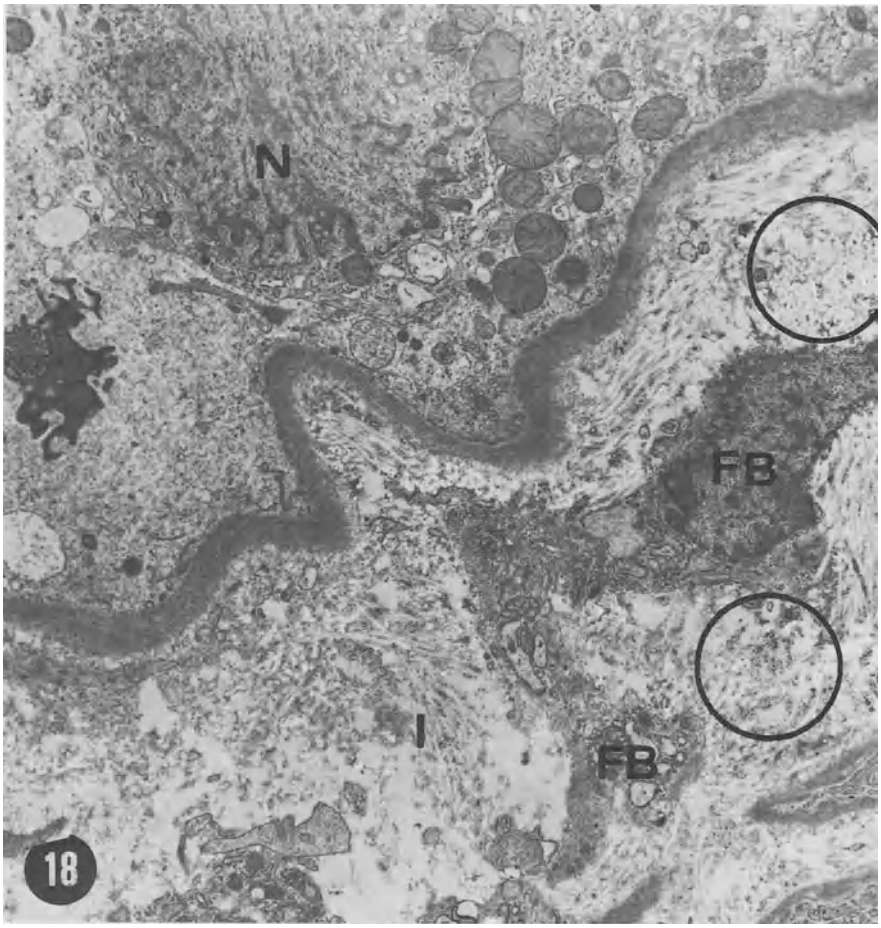

Fig. 11-18. Electron microscopy of a tubule and the interstitium from the patient B.B. (see Fig. 11-11), whose glomerular EM was shown in Fig. 11-14. The tubule reveals atrophy of the nucleus (N), the presence of a few mitochondria, and very slight cytoplasmic constituents. The basement membrane is curled up and electron-dense. The interstitium (I) demonstrates many fibroblasts (FB) and bundles of collagen fibers (circles) (UA + LC, ×10,000).

SUMMARY

1. Patients presenting with microscopic hematuria and asymptomatic proteinuria are commonly referred to nephrologists.

2. In most cases, urological workups fail to demonstrate anatomical lesions to account for the hematuria.

3. By and large, most of these cases undergo renal biopsy procedure.

4. Renal tissue studied by light microscopy (LM) alone or in combination with immunofluorescence microscopy (IFM) appears to be nondiagnostic in most cases.

5. Electron microscopy (EM) study alone or in combination with IFM study constitutes the most important diagnostic aid in the syndrome of microscopic hematuria, asymptomatic proteinuria, and "focal glomerulonephritis." With the use of EM, one-third of the total number of patients studied reveal normal or nondiagnostic morphology; two-thirds show definite abnormalities.

6. There is an increasing tendency to refer to this syndrome as IgA glomerulonephritis or Berger's disease. The glomerular mesangial changes (EM) similar to those in Berger's disease have been observed without demonstrable IgA deposit. Therefore, the cause and effect relationships among the IgA deposit, ultrastructural mesangial changes, and the clinical syndrome of hematuria are still unclear. No etiological factors have yet been advocated for this syndrome.

7. With the possible exception of hereditary nephritis, the histopathological studies by numerous individuals reveal consistently mild, nonspecific, or residual renal lesions. In general, these types of renal lesions are either reversible or nonprogressive.

8. The electron microscopy study of renal biopsy in hereditary nephritis may serve as a useful tool to predict evolution of the disease in the asymptomatic family members of patients with hereditary nephritis.

9. Renal biopsy procedure and studies of renal tissue using LM, EM, and IFM are recommended to rule out progressive diseases, e.g., lupus nephritis, mesangioproliferative glomerulonephritis. Caution must be exercised to avoid the inadvertent diagnosis of focal glomerulonephritis without substantial documentation.

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12 Accomplishments of Electron Microscopy Study in the Practice of Nephrology

PART 1
STUDIES OF GLOMERULI AND TUBULES IN GLOMERULAR AND TUBULOINTERSTITIAL DISEASES

INTRODUCTION

The practice of nephrology (renal medicine) involves mainly those patients with the nephrotic syndrome, the acute syndrome of diffuse proliferative glomerulonephritis, acute uremia, chronic uremia, asymptomatic proteinuria, recurrent microscopic hematuria, and hypertension. A safe method of obtaining a 1- to 2-cm core of renal tissue by means of closed needle biopsy and the increasing availability of electron microscopy (EM) techniques for evaluation of the renal tissue have popularized EM study among clinicians and pathologists in the practice of nephrology. There is little argument concerning the value of EM of renal tissue in patients with the nephrotic syndrome, but controversies still exist as to whether or not EM study is of clinical benefit in other renal diseases.

Over the last two decades, EM of renal tissues has been done extensively in the United States and several other countries. The yield of EM compared to light microscopy (LM) study of renal tissues has been assessed by several individuals including the author. Muehrcke and colleagues (1969) were the first to assess the value of EM study of renal biopsies in the practice of nephrology and report the results. The results of EM studies of renal biopsies from 179 patients with a variety of renal diseases were analyzed and are shown in Table 12-1.

Normal kidney tissue was obtained from two patients who underwent abdominal surgery for other purposes. It was concluded at that time that EM significantly contributed to the diagnosis of diabetic glomerulosclerosis, acute tubular necrosis, membranous glomerulonephritis (GN) due to renal vein thrombosis, and amyloidosis. From this analysis, EM study was thought to be very valuable in distinguishing membranous GN due to renal vein thrombosis from idiopathic membranous GN and lupus membranous GN. Also, in this analysis EM study was found to contribute to the diagnosis of poststreptococcal GN in four patients who failed to reveal bacteriologic or serologic evidence of a preceding streptococcal infection. Typical "humps" were found, thus supporting the diagnosis of poststreptococcal glomerulonephritis. In this study, an important contribution of EM was the diagnosis of glomerulosclerosis as the cause of asymptomatic proteinuria in the prediabetic patients. This knowledge permitted us to predict that frank diabetes mellitus would ensue later on. However, the 6% (11 of 179 patients) yield of the EM study in this analysis raised some doubts as to the usefulness of EM study as a routine procedure in the practice of nephrology.

Four years later, Siegel *et al.* (1973) published the results of an assessment of EM study in the examination of renal biopsies. In this study, 213 consecutive renal biopsy specimens were prepared for both LM and EM during a period of 28 months. Since these specimens were examined independently by two renal pathologists, one with and the other without the benefit of the ultrastructural studies, the study was better able to evaluate the role of EM in the routine examination of renal biopsies. Of 213 biopsy specimens processed, 27 were inadequate for LM and/or EM study. In 22 of the remaining 186 biopsy specimens (11%), a substantially different diagnosis was suggested by the independent eval-

Table 12-1
Clinical Diagnosis of 179 Patients in Whom Renal Biopsies Were Studied by Electron Microscopy

Clinical diagnosis	Number of patients
Nephrotic syndrome	45
Acute renal failure	23
Hypertension	22
Acute hemorrhagic glomerulonephritis	19
Benign recurrent hematuria	15
Chronic renal failure	12
Diabetes mellitus with proteinuria	10
Persistent asymptomatic proteinuria	11
Systemic lupus erythematosus	8
Hypokalemic nephropathy	8
Miscellaneous renal disease	6
Normal	2

Source: From Muehrcke *et al.* (1969), by the kind permission of the editor.

uation with EM. A comparison of the diagnoses made by LM study alone and those by combined LM and EM study is shown in Table 12-2. The clinical manifestations in these 22 patients included nephrotic syndrome, 14; proteinuria, 5; homograft rejection, 2; and nephritis of unknown cause, 1. These authors concluded that EM study was most useful (1) in distinguishing lipoid nephrosis from focal glomerular sclerosis, (2) in the diagnosis of the early stage of membranous nephropathy, and (3) in differentiating the recurrence of lipoid nephrosis from a modified rejection reaction in homograft transplantation. The management and specific therapy in these conditions were affected by the diagnosis established on the basis of EM study of renal biopsies. These authors also found EM study to be valuable in the determination of the activity of the pathological process, lupus glomerulonephritis in particular.

DETAILED ANALYSIS OF ACCOMPLISHMENTS

A decade after the initial analysis, we again reported a critical analysis of the accomplishments of EM in the practice of nephrology (Mandal *et al.*, 1979). This analysis was undertaken with the following intentions: (1) to evaluate the extent of disparities between LM and EM diagnoses, (2) to determine the exact benefits (absolute and relative values) of EM in individual groups of renal diseases, and (3) to illustrate certain examples to document these values.

The results of renal biopsy studies from 72 patients were included in this analysis. The patients were broken down into three groups according to clinical diagnosis obtained from histopathology slips provided with the biopsy specimens. These groups included nephrotic syndrome (39 patients), diffuse proliferative glomerulonephritis (15 patients), and focal glomerulonephritis including asymptomatic proteinuria and recurrent hematuria (18 patients). The LM studies were done in semiblind fashion by the staff members of the department of pathology,

Table 12-2
Biopsies in Which Electron Microscopy Led to a Different Diagonsis Than That Made by Light Microscopy Alone

Light microscopy	Light and electron microscopy	Number of specimens
Mild or chronic glomerulonephritis	Lipoid nephrosis with focal sclerosis	12
Focal or mild glomerulonephritis	Early membranous nephropathy	4
Focal glomerulonephritis	Lipoid nephrosis	1
Transplant rejection	Lipoid nephrosis (recurrent)	1
Proliferative and membranous GN	Acute postinfectious GN	1
Acute postinfectious GN	Anaphylactoid nephritis	1
Chronic active GN	Myeloma	1
Transplant rejection	Acute tubular necrosis	1
Total		22

Source: From Siegel *et al.* (1973).

"semiblind" meaning that the initial assessment was made by studying sections stained with H & E without prior knowledge of the clinical materials. If the morphological diagnosis was different than that suspected from the clinical presentations, another study of the biopsy was made, using special stains.

An analysis of the results of this study reveals the following:

1. It resolved nine different types of glomerular pathology among the biopsies submitted for this specialized study, including (a) proliferative glomerulonephritis, (b) membranous glomerulonephritis, (c) mesangioproliferative glomerulonephritis (synonyms: mesangiocapillary GN, mesangiomembranous GN), (d) foot process fusion disease (lipoid nephrosis), (e) sclerosing glomerulonephritis (focal glomerular sclerosis), (f) hereditary nephritis, (g) glomerulosclerosis, (h) amyloidosis, (i) nonspecific histopathology.
2. The distinction between glomerulonephritis and glomerulosclerosis can easily be made by EM (see Table 6-1). This is important since mistaking one for the other is not uncommon using LM study alone.
3. A diagnosis of membranoproliferative or mixed membranous and proliferative GN is frequently made by LM study. This LM entity, in the experience of the author, has often been resolved by EM to be another disorder, most frequently idiopathic membranous glomerulonephritis, less often focal glomerular sclerosis, and rarely proliferative glomerulonephritis.

ANALYSIS OF VALUE

The value of EM study of renal tissue has been assessed as either "equivalent" or "disparate." The term *equivalent* is used when LM has made a diagnosis and EM has corroborated or confirmed the LM diagnosis. The term *disparate* refers to: (1) "absolute value," when LM has revealed no change or minimal change but EM has made the diagnosis, or (2) "relative value," when LM has made a diagnosis but EM has altered the LM diagnosis and/or defined the severity of the disease process. The details of equivalent and disparate diagnoses in nephrotic syndrome, diffuse proliferative glomerulonephritis, and focal glomerulonephritis are listed in Tables 12-3 through 12-5. The percentages of equivalent and disparate EM diagnoses in all these groups of glomerular diseases and the significance of the differences are shown in Table 12-6, where it can be seen that disparity occurs twice and almost five times as much as equivalency in the nephrotic syndrome and focal glomerulonephritis, respectively, whereas the occurence of disparity is one-half that of equivalency in diffuse proliferative glomerulonephritis. The differences were highly significant in nephrotic syndrome and focal glomerulonephritis, but no difference was observed in diffuse proliferative glomerulonephritis.

The absolute and relative values of EM studies in nephrotic syndrome, diffuse proliferative glomerulonephritis, and focal glomerulonephritis were sub-

Table 12-3
Breakdown of Equivalent and Disparate Diagnosis between LM and EM in Nephrotic Syndrome

	Light microscopy			Electron microscopy		
Group	Diagnosis	Total number	Equivalent	Disparate diagnosis	Total number	Individual number
1	Membranous glomerulonephritis	6	6		0	0
2	Membranoproliferative	11	0		11	
				Membranous glomerulonephritis		6
				Glomerulosclerosis		2
				Focal glomerular sclerosis		1
				Mesangioproliferative glomerulonephritis		2
3	Normal, compatible with lipoid nephrosis or nondiagnostic	9	0		9	
				Lipoid nephrosis (active)		5
				Lipoid nephrosis (remission)		2
				Immune complex glomerulonephritis		1
				Focal glomerular sclerosis		1
4	Chronic pyelonephritis	4	4	Glomerular changes are consistent with lipoid nephrosis	0	0
5	Chronic glomerulonephritis	1	0	Idiopathic membranous glomerulonephritis	1	1
6	Focal glomerular sclerosis	4	2	Resolution of lipoid nephrosis	2	2
7	Lupus nephritis	1	1		0	0
8	Advanced nephrosclerosis	1	0	Mesangioproliferative glomerulonephritis	1	1
9	Increase in mesangial matrix	2	0		2	
				Membranous glomerulonephritis		1
				Focal glomerular sclerosis		1
Total		39	13		26	26

Source: From Mandal *et al.* (1979), by the kind permission of the series editors.

Table 12-4
Breakdown of Equivalent and Disparate Diagnosis between LM and EM in Diffuse Proliferative Glomerulonephritis

	Light microscopy			Electron microscopy		
Group	Diagnosis	Total number	Equivalent	Disparate diagnosis	Total number	Individual number
1	Acute proliferative glomerulonephritis	4	3 (acute poststreptococcal)	Rapidly progressive glomerulonephritis	1	1
2	Diffuse proliferative glomerulonephritis	7	5 (immune complex)	Hereditary nephritis	2	2
3	Hereditary nephritis	1	1		0	0
4	Glomerulonephritis	1	1		0	0
5	Focal glomerular sclerosis[a]	1	0	Nonspecific	1	1
6	Acute poststreptococcal glomerulonephritis	1	0	Rapidly progressive glomerulonephritis	1	1
Total		15	10		5	5

[a] Sclerosing glomerulonephritis.
Source: From Mandal *et al.* (1979), by the kind permission of the series editors.

Table 12-5
Breakdown of Equivalent and Disparate Diagnosis between LM and EM in Focal GN

	Light microscopy			Electron microscopy		
Group	Diagnosis	Total number	Equivalent	Disparate diagnosis	Total number	Individual number
1	No diagnostic abnormalities	3	1		2	
				Hereditary nephritis		1
				Extracapillary proliferative glomerulonephritis		1
2	Inadequate specimen	1	0	Focal glomerular sclerosis	1	1
3	Normal	1	0	Lipoid nephrosis	1	1
4	Focal glomerular sclerosis	2	1	Segmental glomerulosclerosis	1	1
5	Glomerulosclerosis	2	0		2	
				Normal kidney		1
				Rapidly progressive glomerulonephritis/ TTP		1
6	Focal proliferative glomerulonephritis (or minimal change)	5	0		5	
				Healing stage of poststreptococcal or early stage of mesangioproliferative glomerulonephritis		1
				Mesangioproliferative glomerulonephritis		1
				Segmental glomerulosclerosis		1
				Nonspecific		1
				Hereditary nephritis (early stage)		1
7	Diffuse proliferative glomerulonephritis	2	1	Mild rapidly progressive glomerulonephritis	1	1
8	Acute and chronic glomerulonephritis	1	0	Mild rapidly progressive glomerulonephritis	1	1
9	Increase in cells and matrix	1	0	Hereditary nephritis (early stage)	1	1
Total		18	3		15	15

Source: From Mandal *et al.* (1979), by the kind permission of the series editors.

Table 12-6
Significance of EM Diagnosis

Clinical diagnosis	Total number of patients	LM vs. EM	Percentage	p value
Nephrotic syndrome	39	Equivalent = 13	33.0	
		Disparate = 26	67.0	<0.02
Diffuse proliferative glomerulonephritis	15	Equivalent = 10	66.66	
		Disparate = 5	33.33	>0.05
Focal glomerulonephritis	18	Equivalent = 3	16.66	
		Disparate = 15	83.33	<0.001

Source: From Mandal *et al.* (1979), by the kind permission of the series editors.

jected to critical analysis, and the results are shown in Table 12-7. Again, it can be observed that the proportion of disparate diagnosis is twice and almost five times as much as that of equivalent diagnosis in nephrotic syndrome and focal glomerulonephritis, respectively. Using McNemar's test, the nine patients with nephrotic syndrome were found by EM to have lipoid nephrosis in seven cases, immune complex glomerulonephritis in one case, and focal glomerular sclerosis in one case (which was missed by LM). This yield of EM is highly significant (p <0.01). For focal glomerulonephritis, the proportion of sole diagnosis (absolute value) is the same as for nephrotic syndrome, but the sample size is too small to obtain statistical significance.

EXAMPLES OF DISPARATE DIAGNOSIS

Patient #1.* C. M., a 26-year-old white male, was referred to Oklahoma City VA Hospital in July 1972, for investigation of repeated episodes of coke-colored urine. His past health has been essentially normal. Physical examination was normal. Laboratory studies—Hematocrit 39%, BUN 19 mg/100 ml, serum creatinine 1.1 mg/100 ml, 24-hr endogenous creatinine clearance 95 ml/min, 24-hr proteinuria 1.5 g, normal electrolytes, normal platelet count, and normal coagulation profile. A percutaneous renal biopsy was performed and the renal tissue was studied by both LM and EM. LM revealed normal to mildly hypercellular glomeruli; EM showed marked changes in the glomeruli and interstitium. The glomerular changes were characterized by irregularity, fragmentation, and splitting of the glomerular basement membrane (GBM), excessive formation of basement membrane (BM)-like materials, atrophy of endothelial and epithelial cells and occasional neutrophils within the lumen of glomerular capillaries. There was complete absence of electron-dense deposits. The interstitium was pronounced due to the presence of an excessive number of plasma cells, fibroblasts, and collagen fibers (Fig. 12-1; also see Figs. 8-14 to 8-18). A diagnosis of hereditary nephritis was suggested, and further questioning revealed history of glomerulonephritis in family members.

He was lost to follow-up until January 1977 when he was admitted for the second

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Table 12-7
Values of EM Studies in Glomerular Diseases[a]

1	2		3		4		5		6		7
	Both positive on disease condition and agree on anatomic pathology		Both positive on disease condition but disagree on anatomic pathology[b]		EM positive, LM negative on disease condition (sole diagnosis)[c]		LM positive, EM negative on disease condition		Both negative on disease condition		
Disease condition	No.	Proportion	No.	Proportion	No.	Proportion	No.	Proportion	No.	Proportion	Total
Nephrotic syndrome	13	0.33	17	0.45	9	0.22	0	0	0	0	39
Diffuse glomerulonephritis	10	0.67	5	0.33	0	0	0	0	0	0	15
Focal glomerulonephritis	3	0.17	10	0.56	4	0.22	0	0	1	0.06	18

[a] Column 2 is equivalent diagnosis; columns 3 and 4 are disparate diagnoses.
[b] Relative value.
[c] Absolute value.
Source: From Mandal *et al.* (1979), by the kind permission of the series editors.

time with complaints of easy fatigability, occasional nosebleeds, and bloody urine. He had blood pressure of 150/90 mm Hg; otherwise his physical examination was normal. Urinalysis: 3+ protein, numerous RBC/HPF, also unstated number of hyaline, granular, and white cell casts. A 24-hr creatinine clearance was 12 ml/min and the serum creatinine was 7.8 mg/100 ml. He was also found to have massive proteinuria (3.9 g/24 hr).

Comments. The glomerular changes observed by EM in this patient are consistent with hereditary nephritis. This pathological process generally progresses relentlessly in male subjects who culminate in chronic renal failure between the second and third decades. The glomerular changes characterizing the early stage of hereditary nephritis have been reported by other authors. This report cites an example in which LM study failed to unravel the condition, but EM study suggested the proper diagnosis. This helped the clinicians to determine the future course and to plan appropriate management for this patient. Excessive proteinuria in the range of nephrotic syndrome has been reported to occur in several families with hereditary nephritis. The mechanism of this heavy proteinuria is unknown.

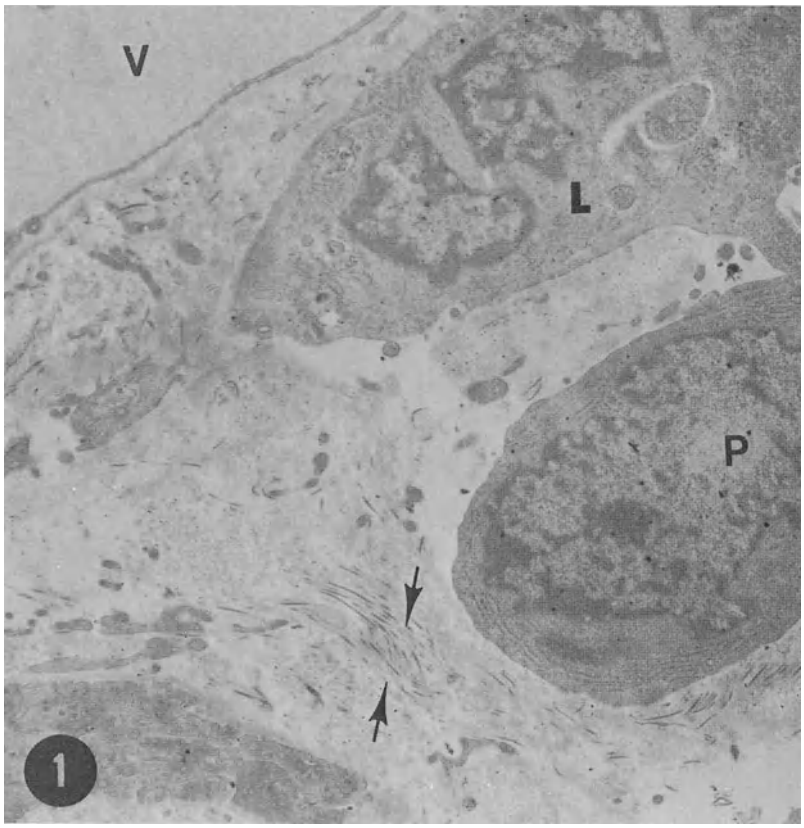

Fig. 12-1. Infiltration of the interstitium by lymphocyte (L), plasma cell (P), and collagen fibers (opposing arrows) is a conspicuous feature. A vein (V) is seen (UA + LC, ×10,000).

Patient #2.* F.S., a 57-year-old white male, was admitted to Oklahoma City VA Hospital in January 1973, for investigations of proteinuria and hypertension. His 24-hr proteinuria ranged from 5 to 9 g, and an inulin clearance ranged from 40 to 42 ml/min. His blood pressure ranged from 180 to 190/110 to 120 mm Hg. He had a percutaneous renal biopsy which was studied by both LM and EM. Advanced membranous glomerulonephritis was found (Fig. 6-28) by LM and was confirmed by EM to be stage 3 idiopathic membranous glomerulonephritis (Fig. 6-33). He was placed on cyproheptadine (periactin) for 1 year with no significant change in the severity of proteinuria. He is currently receiving antihypertensive drugs and diuretics.

Comments. This patient is an example in which LM study is equivalent to EM study for diagnosis. The EM study established correct staging (severity of disease) of membranous glomerulonephritis, which has enabled us to determine the irreversible stage of his disease. Nonresponsiveness of this stage of membranous GN to any therapy has been reported by other investigators.

Patient #3.* F.W., a 57-year-old Indian male, was admitted to Oklahoma City VA Hospital in April 1974, with a 3-week history of increasing pedal edema, orthopnea, and dyspnea on exertion. The other pertinent information included a 3-year history of insulin-dependent diabetes mellitus. Admission physical examination revealed blood pressures of 160/100 mm Hg, venous distension in the neck, and pedal edema. Laboratory studies included serum creatinine 1.7 mg/100 ml, BUN 57 mg/100 ml, and 24-hr proteinuria 6.2 g. Several urinalyses showed persistent pyuria but no growth of microorganisms on culture (without antibiotic). A percutaneous renal biopsy was done and the renal tissue was studied by LM and EM. LM showed diffuse proliferative glomerulonephritis with exudation (Fig. 12-2); EM revealed typical "humps," slight proliferative change, and moderate exudation of neutrophilic leukocytes in the glomeruli (Fig. 12-3). Retrospective questioning with regard to sore throat or upper respiratory tract illnesses 2 to 3 weeks prior to hospital admission gave negative answers. ASO titer was normal and the serologic tests for SLE and syphilis were nonreactive. Serum C3 was less than 50 mg/100 ml. Repeated blood cultures failed to grow any organisms. Three years later, he has no edema, decreased proteinuria (2.1 g/24 hr), persistently low serum C3 (108 mg/100 ml), and normal renal function.

Comments. This patient demonstrates several points of clinical interest: (1) The kidneys in patients with diabetes mellitus are not immune from primary glomerular diseases. Typical "humps" argued in favor of poststreptococcal glomerulonephritis or glomerulonephritis of subacute bacterial endocarditis (SBE). These diagnoses are supported by very low serum C3. SBE is the most unlikely possibility because of complete lack of clinical and bacteriological evidence of SBE. In addition, some improvements in clinical picture and laboratory studies over a period of 3 years tend to rule out SBE. Poststreptococcal glomerulonephritis with acute renal failure, acompanied by elevation of ASO titer and low serum C3 has been reported in a 46-year-old woman with 17 years of diabetes mellitus. (2) Congestive cardiac failure observed in this patient was attributed to

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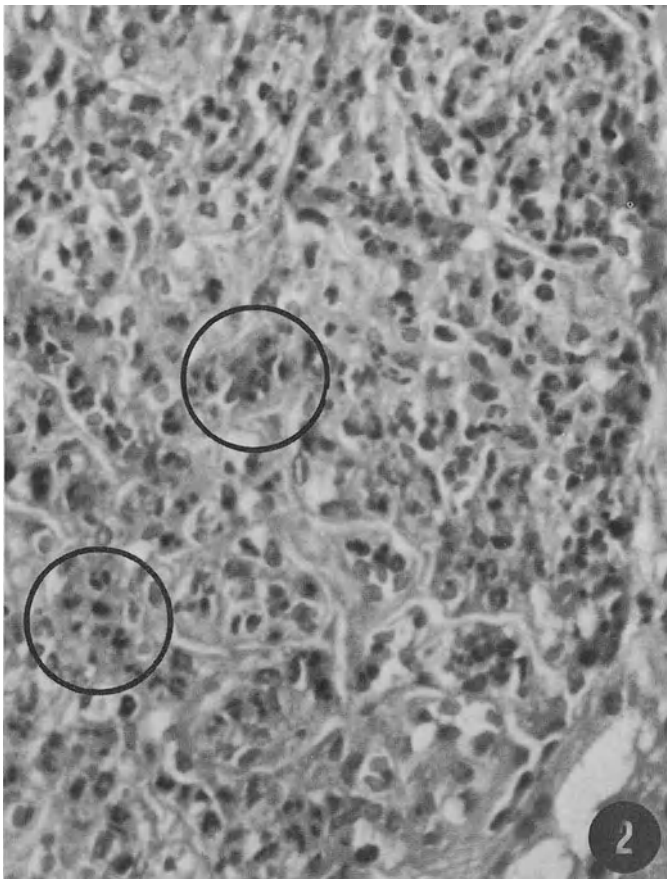

Fig. 12-2. Enlargement of the glomerulus due to marked proliferative and exudative changes. Pronounced exudation of polymorphonuclear leukocytes (circles) is shown (H & E, ×400).

fluid retention as a result of sharp decrease in GFR. This also might explain acute renal failure in a similar patient reported by other authors. (3) It has been mentioned earlier in this chapter that we, in an earlier study, documented post-streptococcal GN in four patients, all of whom had negative bacteriological and serological evidence for streptococcal infection.

Patient #4.* B.W., a 27-year-old white housewife, was first admitted to Oklahoma Medical Research Foundation in September 1973, for evaluation of hypokalemic alkalosis and hyperuricemia. She experienced acute flank pain in 1966 and was told that she probably had a kidney stone. Over the following 5 years, she has had one to two episodes of urinary tract infections every year; these episodes were treated empirically with antibiotics without confirmation of the diagnosis. In 1971, hyperuricemia was detected and she was started on allopurinol. One year prior to admission, she began to experience

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generalized weakness and tiredness. Admission physical examination was essentially normal. She had a percutaneous renal biopsy and the renal tissue was studied by LM and EM. LM study was essentially normal (Fig. 12-4); EM study of glomeruli revealed no abnormality other than slight tortuosity of the peripheral capillary loops. Tubules showed electron-dense and irregularly thickened basement membranes, wide separation of the tight junction of the cells, but no evidence of mitochondrial damage or necrosis of cells. The interstitium was prominent and characterized by edema (electron-lucent areas), fibroblasts, and a moderate amount of collagen tissue (Fig. 12-5). A diagnosis of acute tubulointerstitial nephritis was made. The tubular morphology was consistent with severe hypokalemia and the interstitial change could result from persistent hyperuricemia.

Comments. The failure of LM study to demonstrate mild tubulointerstitial changes such as occurred in this patient has interfered with our ability to study the relationships between metabolic derangements and renal morphological changes. The effectiveness of triamterene to reverse hypokalemia and alkalosis denies, at least, tubulointerstitial abnormalities as primary to hypokalemia. The passage of excessive amounts of uric acid due to hyperuricemia and hyperuricosuria across the basement membranes of medullary and papillary tubules during concentration of urine may partly explain the tubulointerstitial changes. This idea is supported in part by the observations of Heptinstall (1974), who stated in his description of renal lesions in gout that the walls of the tubules are frequently deficient and that the deposits appear to be in the interstitial tissue, a rim of giant cells and other mononuclear cells usually being present in relation to the crystalline material.

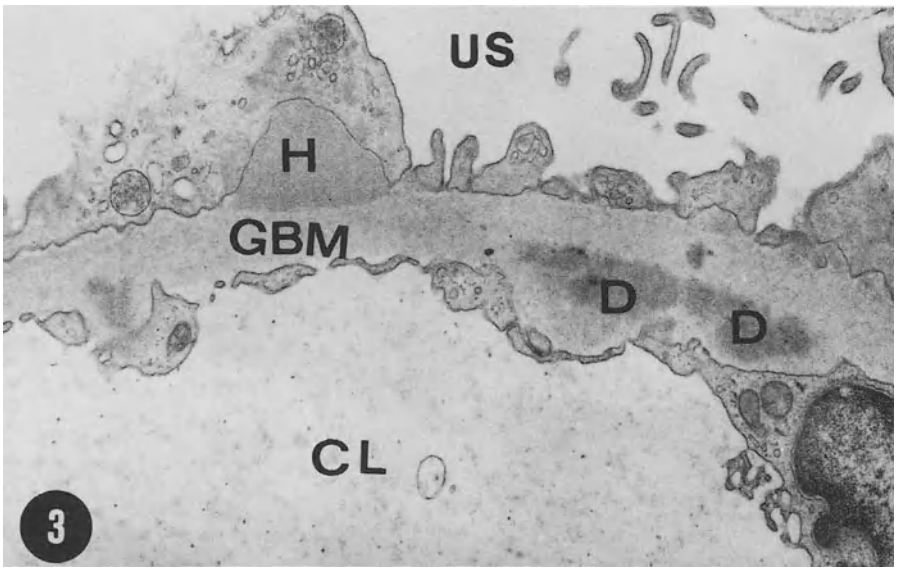

Fig. 12-3. A typical "hump" (H) is shown. The glomerular basement membrane (GBM) is within normal limits. Note electron-dense deposits (D) within the GBM. Urinary space (US) and lumen of the capillary (CL) are shown (UA + LC, ×12,000).

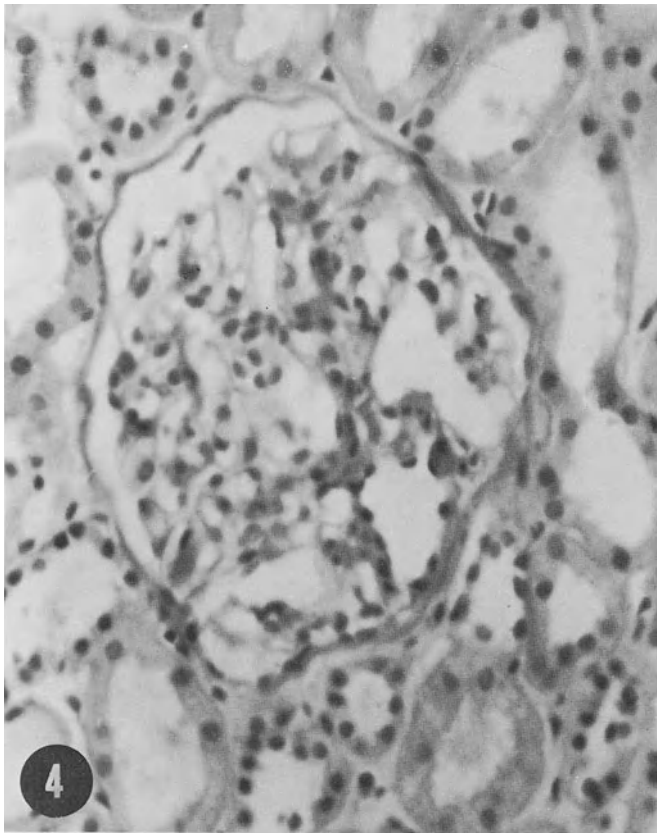

Fig. 12-4. The glomerulus appears essentially normal. Some tubules show swelling of the cells, whereas others reveal hyperchromatic nuclei (H & E, ×200).

Patient #5.* H.B., a 1-month-old premature male, was admitted to Oklahoma Children's Memorial Hospital for investigation of edema and 4+ proteinuria. An open renal biopsy was done and the renal tissue was studied by LM, EM, and immunofluorescence microscopy (IFM). Details of the clinical and renal histology studies have been reported in Chapter 7. In this chapter, the value of EM study of this condition is emphasized by the striking and varied types of abnormalities in the glomeruli, the cystic changes in the proximal tubules with normal distal convoluted tubule and loop of Henle, and cystic changes in the small arteries and arterioles (see Figs. 7-18 to 7-21). These EM changes contrasted with LM findings.

Comments. The main purpose of discussing this patient is to demonstrate that glomerular changes other than the commonly observed thin GBM and fusion of foot processes may be found in congenital nephrotic syndrome; these changes include splitting of the GBM and the presence of linear deposits. A combination of these findings suggests an anti-GBM antibody type of reaction. These glomerular changes, along with the widespread arterial vascular changes, may explain

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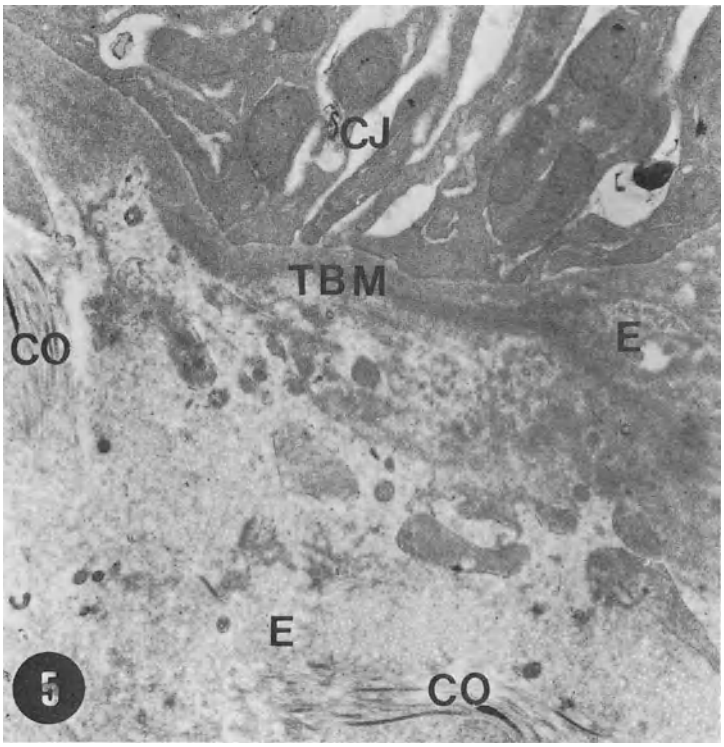

Fig. 12-5. A tubule shows electron-dense basement membrane (TBM). Marked edema (E) of a part of TBM is observed. Swelling of plasma membrane infoldings has caused wide separation of the cell junctions (CJ). Pronounced edema (E) and collection of collagen fibers (CO) in the interstitium are seen (UA + LC, ×7000).

rapid worsening of the renal functions in most of the children with congenital nephrotic syndrome. The child died within a year after his first hospital admission.

Patient #6.* S.P., a 20-year-old white female, was well until December 1974, when she developed migratory polyarthralgia. She was admitted to Presbyterian Hospital of Oklahoma City for investigation. Physical examination was unremarkable. Laboratory studies—WBC 3500, positive LE cell and ANA; urinalysis: 60 to 80 RBC and 8 to 12 WBC/HPF. A 24-hr proteinuria was 0.6 g, and a 24-hr creatinine clearance was 47 ml/min. A percutaneous renal biopsy was done in July 1975, and the tissue was studied by LM, EM, and IFM.

LM revealed diffuse proliferative glomerulonephritis; EM of glomeruli revealed marked proliferation of endothelial cells and massive amounts of deep electron-dense deposits in the subendothelial aspect of the GBM (see Figs. 6-13 to 6-15); IFM revealed brilliant granular fluorescence for IgG. She received high doses of methylprednisolone intravenously in an attempt to control the active disease process. She developed severe hypertension and hyperglycemia which led to discontinuation of methylprednisolone. She was then started on alternate-day low-dose prednisone (20 mg) and daily azathioprine (3 mg/kg).

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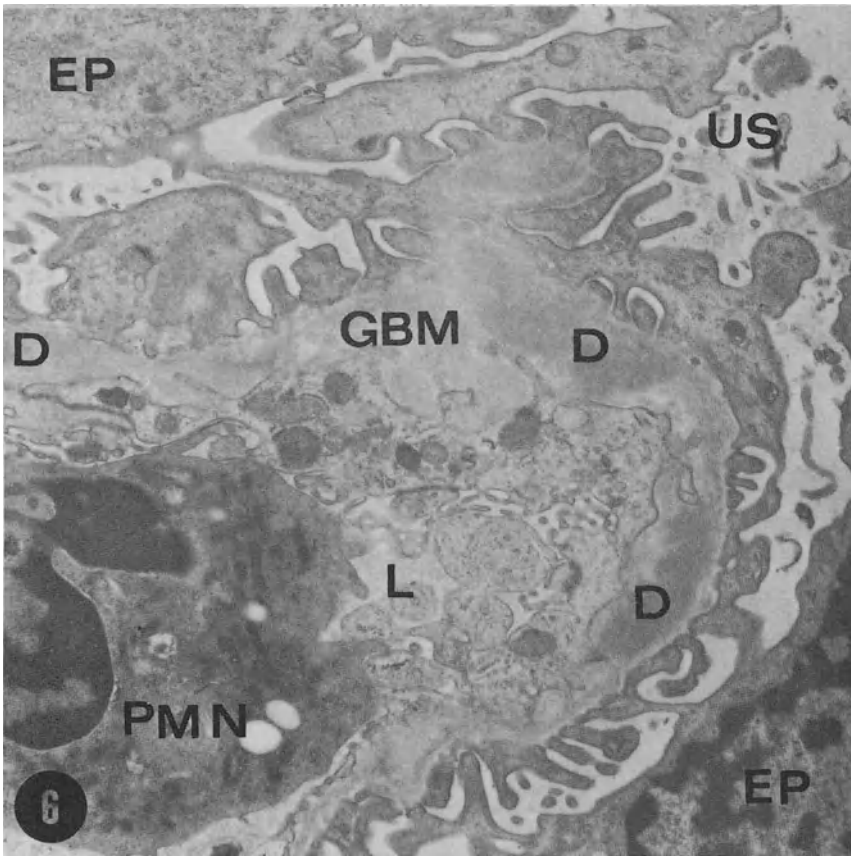

Fig. 12-6. This glomerular capillary exhibits irregularities on the endothelial aspect of the basement membrane (GBM), intramembranous electron-dense deposits (D), and a polymorphonuclear leukocyte (PMN) in the compromised lumen (L). Normal appearing epithelial cells (EP) along with the foot processes and the urinary space (US) are shown (UA + LC, ×17,000).

Follow-up studies demonstrated a decrease in proteinuria and normalization of renal function. At a clinic visit 1 year later, she showed an abrupt increase in proteinuria ranging from 6 to 7 g in 24 hr. However, her renal function remained normal. EM of glomeruli showed mild proliferative change and the absence of subendothelial deposits. This contrasted with the findings of the first biopsy. However, a few spikes and a few extramembranous deposits were found in the GBM (see Fig. 6-18). These findings suggested membranous transformation of lupus proliferative glomerulonephritis. This histological transformation of membranous GN from proliferative GN is supported by the onset of massive proteinuria. She has had persistent proteinuria ranging from 5 to 20 g ever since.

Comments. Even though LM study of the second biopsy showed resolution of the changes observed in the first biopsy, GBM changes characterizing membranous glomerulonephritis (EM) explained the abrupt increase in proteinuria and persistent heavy proteinuria as well.

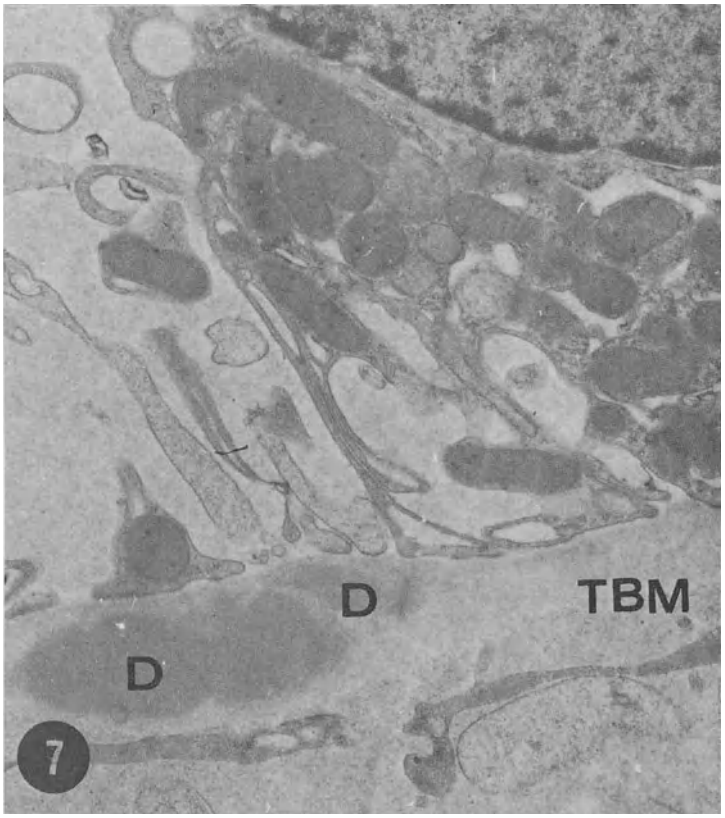

Fig. 12-7. A distal convoluted tubule shows swollen basement membrane (TBM) and discrete electron-dense deposits (D) within TBM. Cells were intact except for separation of the cellular junctions (UA + LC, ×18,000).

Patient #7.* E.G., an 8-year-old white female, was referred to Oklahoma Children's Memorial Hospital in December 1971, with an episode of coke-colored urine. An elevated ASO titer was also reported. In the middle of 1972, she had another episode of coke-colored urine accompanied by an ASO titer of 660 Todd units. She was treated regularly with bicillin. Physical examination was unremarkable. Laboratory studies—Hematocrit 29%, WBC 5000 with adequate platelets, ASO titer 833 Todd units, BUN 27 mg/100 ml, serum creatinine 1.0 mg/100 ml, serum C3 260 mg/100 ml. A percutaneous renal biopsy was performed in January 1973, and the renal tissue was studied by LM and EM. LM study revealed mild endocapillary proliferative glomerulonephritis; EM revealed mild proliferation of endothelial cells, PMN exudation in the glomerular capillaries, discrete intramembranous and subendothelial electron-dense deposits, but the absence of "humps" (Fig. 12-6). Discrete electron-dense deposits were also found in the tubular basement membranes (Fig. 12-7).

She remained well until December 1973, when she developed swelling of the face and was admitted to Oklahoma Children's Memorial Hospital for further evaluation. Admission

* This patient's report has been reproduced from Mandal *et al.* (1979), by the kind permission of the editors of *Pathology Annual.*

physical examination was unremarkable except for the edema. Laboratory studies—BUN 94 mg/100 ml, serum creatinine 8.0 mg/100 ml, urinalysis 2+ protein, 3 to 5 RBC, and 5 to 10 WBC/HPF, serum C3 93 mg/100 ml; ASO titer 125 Todd units. A percutaneous renal biopsy was performed in January 1974, and the renal tissue was again studied by LM and EM.

LM findings were similar to those in January 1973. EM differed, however, showing numerous typical "humps" in addition to the intramembranous deposits (Fig. 12-8). Following this episode, she improved rapidly: her BUN dropped to 34 mg/100 ml and she was discharged 2 weeks after admission. Since then, she has been followed in the renal clinic having, on several occasions, a normal physical examination and laboratory values. At a clinic visit in September 1975, almost 2 years after the second biopsy, she was still asymptomatic and had a serum creatinine of 0.7 mg/100 ml and no abnormality on urinalysis.

Comments. This patient is a perplexing example of recurrent episodes of acute glomerulonephritis. Whether or not each of these episodes was due to poststreptococcal glomerulonephritis is difficult to define. Although recurrent attacks of poststreptococcal GN are uncommon, occasional published and many unpublished observations tend to support the concept that it is not rare. The EM of the first biopsy showed intramembranous and subendothelial deposits while typical "humps" were absent. She recovered 1 year later after the second episode during which the renal biopsy demonstrated typical "humps." The intriguing

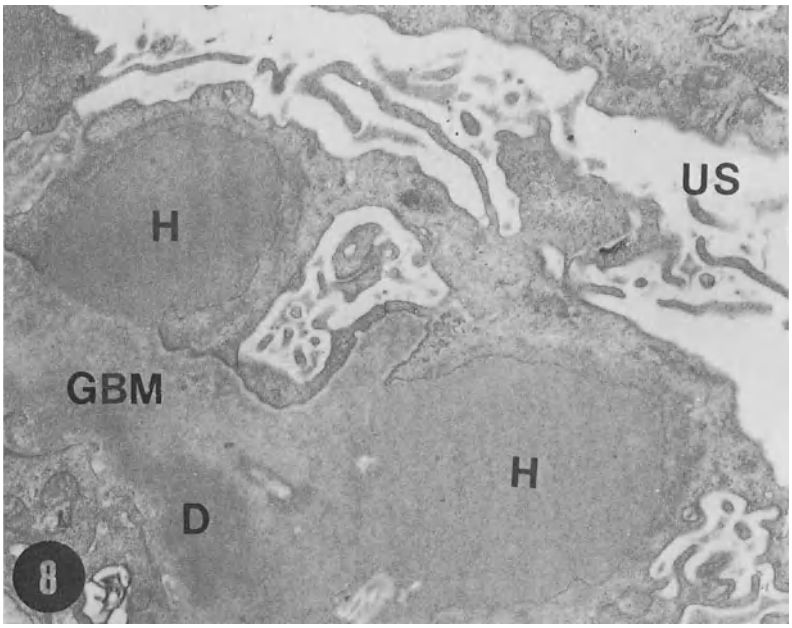

Fig. 12-8. Several typical "humps" (H) are seen in a small portion of the glomerular capillary. Expansion of segments of glomerular basement membrane (GBM) adjacent to humps and electron-dense deposits (D) in these segments are also observed. Urinary space (US) is shown (UA + LC, ×27,000).

feature is the relationship between the intramembranous deposits in the first biopsy and the "humps" in the second biopsy. It is possible that she had recurrent attacks of poststreptococcal glomerulonephritis. Low serum C3 in the second episode is supportive of this view. However, the contradictory findings of high ASO titer during the first admission and a normal ASO titer during the second admission when she was found to have typical "humps" have formed the most intriguing feature of this patient's study.

Patient #8. S.M., a 64-year-old Lebanese male, had numerous hospital admissions beginning in 1965 and continuing until his death on May 12, 1976. His illness apparently started in 1930 when he had albumin in the urine. He showed albuminuria again in 1952. In both instances, albuminuria was considered to be orthostatic. In 1965, he was found to have monoclonal spike by serum protein electrophoresis, which proved to be IgA by immunoelectrophoresis. In August 1973, he presented with edema of the lower extremities and was found to have 7 g protein, mostly albumin, in 24 hr urine. A percutaneous renal biopsy was done and the renal tissue was studied by LM and EM. LM study showed swollen glomeruli with mild hypercellularity especially prominent in the mesangium (Fig. 12-9); EM showed irregular thickening of GBM especially on the endothelial aspect, and expansion of the endothelial aspect of the GBM by electron-lucent and electron-dense

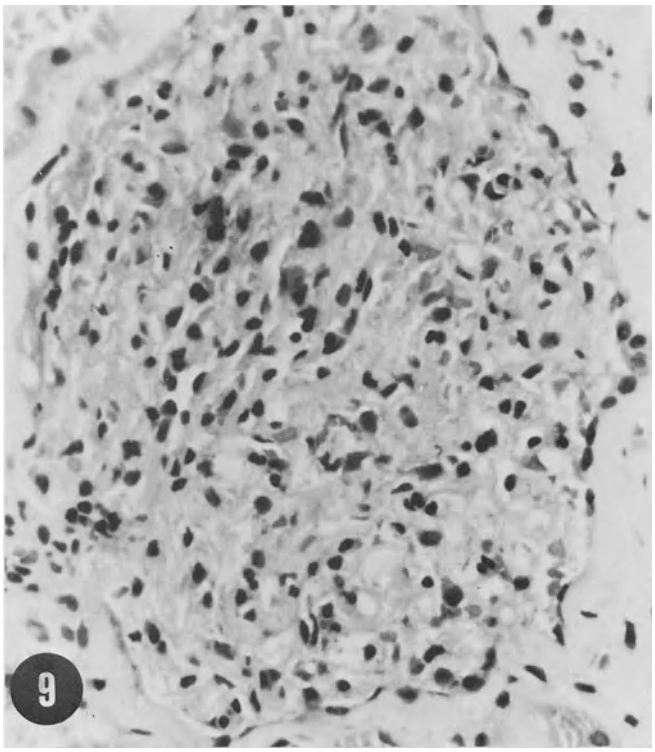

Fig. 12-9. Swollen and mildly hypercellular glomerulus. Hypercellularity is seen more in the mesangium than in the peripheral capiliary loops (H & E, ×400).

materials which contained few nonstriated fibrillar materials (Fig. 12-10). This was more marked in the centrilobular portions of the capillaries than in the peripheral capillary loops. Within GBM, discrete deposits and fibrillar materials could be observed. Tubular basement membranes exhibited irregular thickening and electron-dense deposits in which nonstriated fibrillar materials were discernible (Fig. 12-11). Because of the nonspecific findings on LM and doubtful diagnosis by EM he was treated with corticosteroids for 6 weeks with no change in the severity of proteinuria.

In May 1975, the patient had a second percutaneous renal biopsy and the renal tissue was studied by LM and EM. LM study showed focal glomerular sclerosis and hyalinosis. Congo red staining of the renal tissue and examination with polarizing microscopy showed doubtful birefringence. EM was striking because of the presence of large discrete deposits, especially on the epithelial aspect of the GBM (Fig. 12-12). These deposits were partly electron dense and partly electron lucent and on high magnification clearly demonstrated amyloid fibers (Fig. 12-13). By this time he was spilling up to 35 g protein in 24-hr urine. He developed irreversible renal failure and required hemodialysis. He later died of severe congestive cardiac failure. At autopsy, amyloidosis involving multiple organs was found.

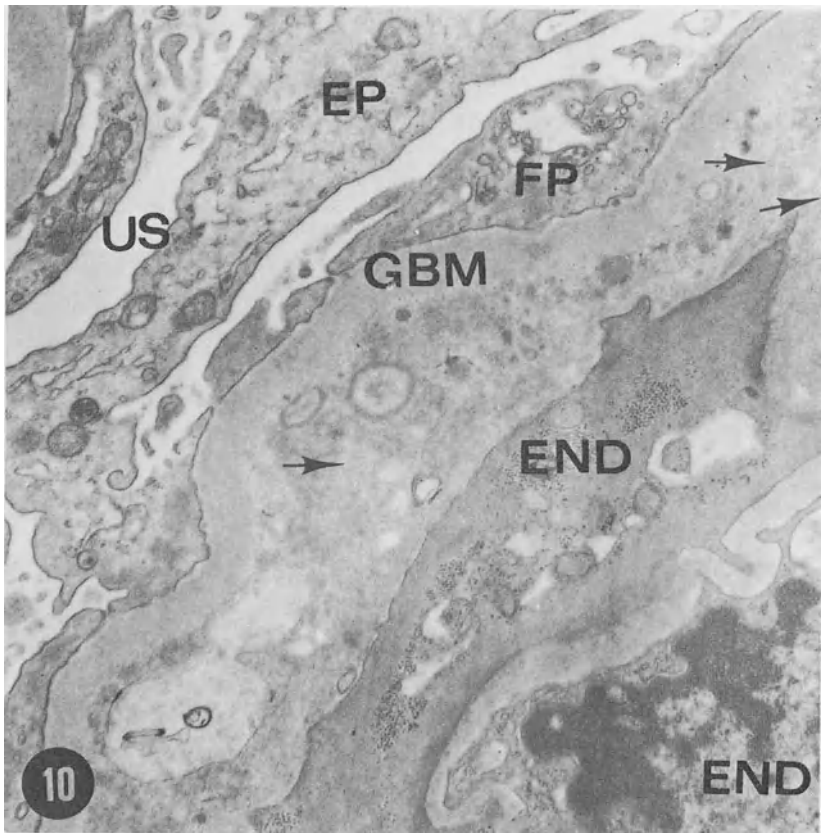

Fig. 12-10. A glomerular capillary demonstrates conspicuous subendothelial space which contains fine nonstriated fibrils (arrows). The glomerular basement membrane (GBM) appears essentially normal. Fusion of foot processes (FP) and partial atrophy of endothelial cell (END) are observed. Epithelial cell (EP) and urinary space (US) are shown (UA + LC, ×22,000).

Comments. This case cites an example of amyloidosis which is rarely preceded by a gammopathy comprising IgA abnormality. Even so, LM study was nondiagnostic. The suspicion of amyloidosis by EM study was aroused without prior knowledge of clinical materials but by the observation of deposits in the tubules in addition to glomeruli. Amyloidosis was confirmed by the EM study of the second biopsy.

VALUE OF EM STUDY

It is now imperative to summarize the value and to provide the reader with a perspective of the role of EM study in the practice of nephrology. The term *value* in reference to this study implies not merely improvements in morphological

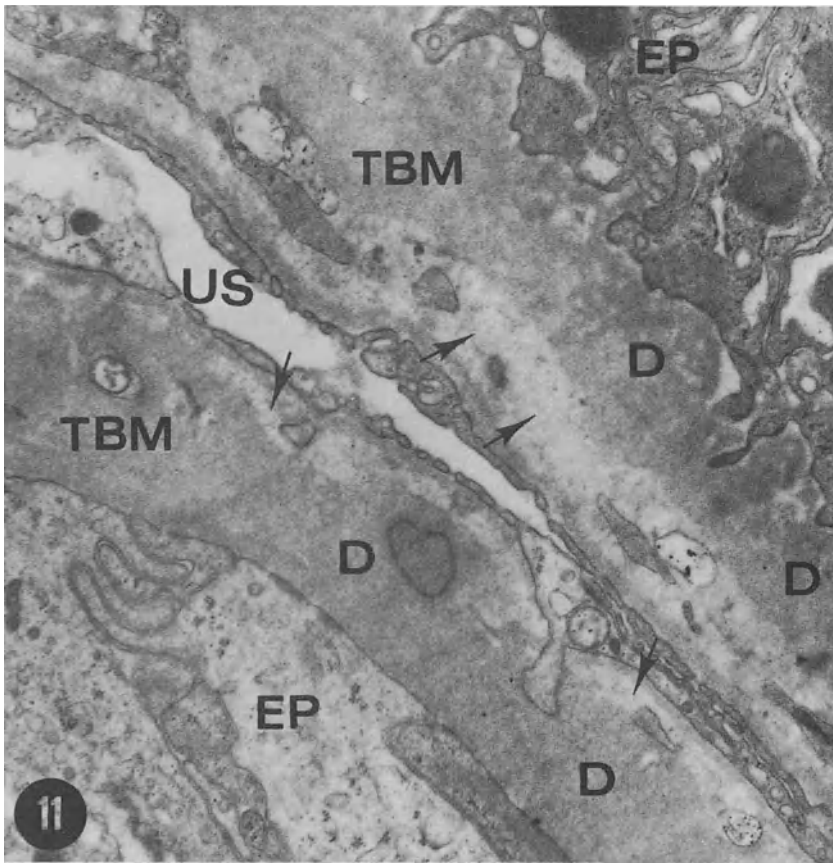

Fig. 12-11. Irregularly thickened and electron-dense basement membrane of the tubules (TBM). The electron density may be due to some type of deposit (D), which in this case was thought to be amyloid on the basis of TBM changes. The epithelial cells (EP) of the tubules appear to be intact. Urinary space (US) is shown (UA + LC, ×20,000).

knowledge, but also guidance to physicians in the practice of nephrology. Attempts have been made to assess this value precisely and to evaluate whether or not the information obtained by the use of this technique is of direct benefit to the patients with renal diseases. For purposes of clarity, value has been divided into absolute, relative, and doubtful or controversial.

Absolute Value

Absolute value is defined as a negative (or normal) LM study and establishment of the anatomical diagnosis by EM study alone. In 39 patients with nephrotic syndrome, LM study in nine was initially reported as normal (Tables 12-3 and 12-7), whereas EM study identified lipoid nephrosis, in an active stage or as undergoing reversibility (partial restoration of foot processes), in seven; immune complex glomerulonephritis in one; and focal glomerular sclerosis in one. This disparity (i.e., the gain of EM study) is highly significant ($p < 0.01$). The absolute yield of EM study in focal glomerulonephritis (including asymptomatic proteinuria and recurrent hematuria) also appears to be significant. In diffuse proliferative glomerulonephritis, EM study does not demonstrate any absolute value (Table 12-6). Therefore, this study emphasizes not merely the known role of EM study in the diagnosis of lipoid nephrosis, but establishes the value of this technique in the study of renal biopsies in nephrotic syndrome and further supports the statement made by other authors. It is especially important not to miss lipoid

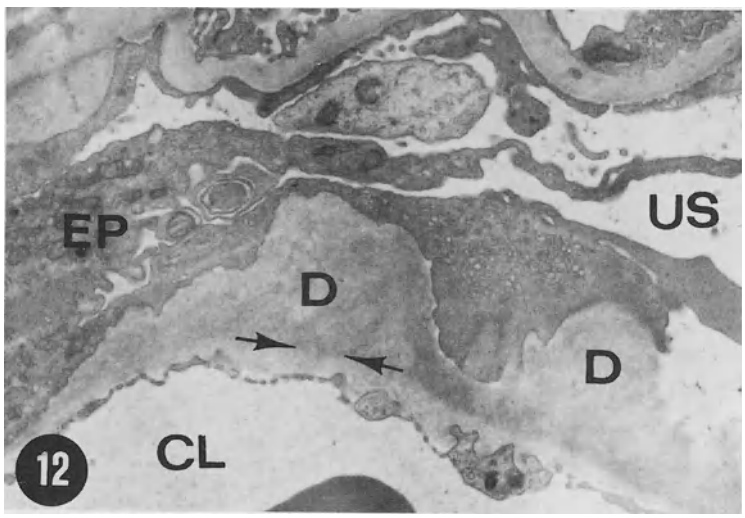

Fig. 12-12. Deposits (D) with an appearance of humps on the epithelial aspect of glomerular basement membrane. A clear demarcation (opposing arrows) is observed between the deposits and the basement membrane. Epithelial cell (EP) and foot processes are fused. Urinary space (US) and capillary lumen (CL) are shown (UA + LC, ×15,000).

nephrosis since it is amenable to specific treatment, and to separate lipoid nephrosis from stage 1 membranous glomerulonephritis. The latter condition may appear normal by LM, especially when viewed early in the course of the disease (Fig. 12-14a).

Nephrotic syndrome has been reported in chronic interstitial nephritis (chronic pyelonephritis) but the cause of heavy proteinuria in this disease has not been determined. Recently, my colleagues and I, using EM, have found glomerular changes consistent with lipoid nephrosis in four patients with chronic interstitial nephritis (chronic pyelonephritis) (Mandal *et al.*, 1977*a*).

The author's analysis supports the importance of EM study of renal biopsy in asymptomatic proteinuria or recurrent hematuria, especially if the LM yield

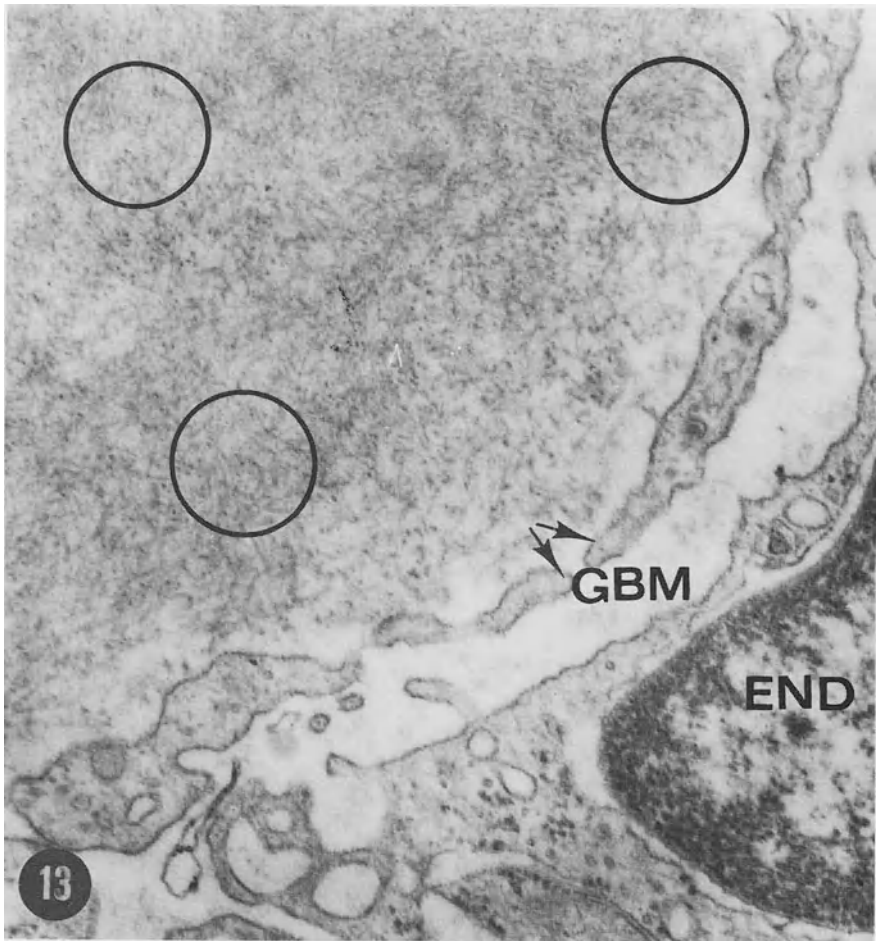

Fig. 12-13. Magnified view of one of the deposits shown in Fig. 12-12 clearly demonstrates a network of amyloid fibers (circles). Foot processes (arrows), glomerular basement membrane (GBM), and endothelial cell (END) are shown (UA + LC, ×38,000).

is slight or none. If the EM is abnormal, these individuals should be followed at least yearly with urinalysis and consecutive renal biopsy studies at 5- to 10-year intervals or earlier if clinical status or laboratory studies or both demand it. Such prospective follow-up studies may unravel the sequence of events which lead to anatomical pathology of "end stage kidney."

Relative Value

Relative value is defined as confirmation of the LM diagnosis by EM study, and further assessment of the activity of the disease process. Several patients' studies have been cited to exemplify the relative values of EM study in the clinical care of patients. Not infrequently, the early stage of hereditary nephritis is missed by LM or misinterpreted as mild proliferative GN. The latter was the

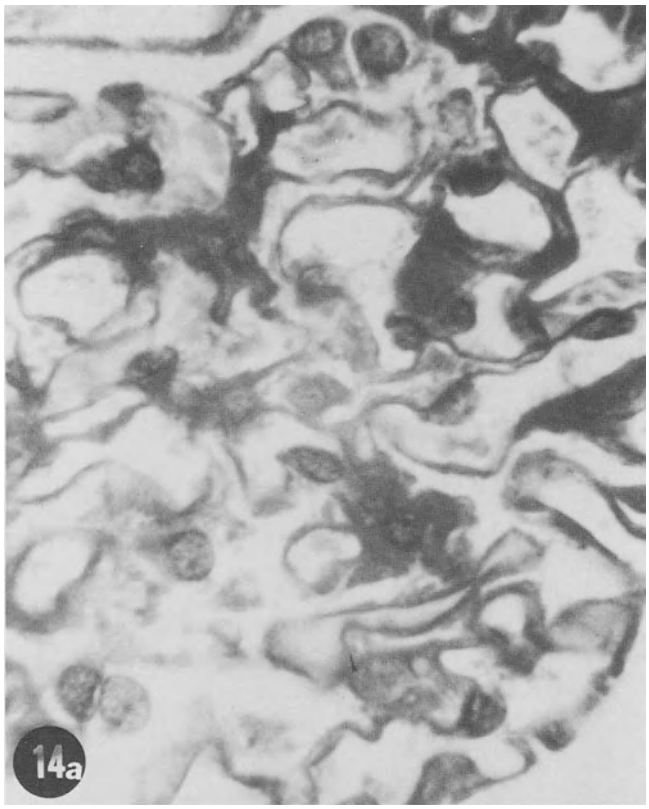

Fig. 12-14. (a) Mild thickening of basement membrane of peripheral capillary loops. An equivocal diagnosis of membranous glomerulonephritis was made (H & E, ×400). (b) Electron microscopy of this glomerular capillary demonstrates numerous spikes (arrows) in the basement membrane (GBM) and a few extramembranous deposits (D). These changes are consistent with stage 3 membranous glomerulonephritis. Lumina (L) of the glomerular capillary and urinary space (US) are shown (UA + LC, ×8200).

diagnosis in patient #1 (C.M.). A diagnosis of hereditary nephritis was made in this patient by EM only. The accuracy of EM diagnosis is supported by the fact that his renal function, which was normal at the time of biopsy, decreased to 12% of normal over a 4-year period. This is difficult to believe from the ambiguous LM findings. Also, it is important to mention that the heavy proteinuria which occurred in this patient might have encouraged the clinician to institute corticosteroids if the EM facilities were unavailable to make the diagnosis of hereditary nephritis. Similarly, EM plays a profound role in the diagnosis of the early stage of amyloidosis when LM study, even with the use of special stains, e.g., congo red or methyl violet, may fail to demonstrate amyloid. The value of LM study in the advanced stage of amyloidosis appears to be equivalent to that of EM study. In patient #4 (B.W.), EM study helped to understand the clinicopathological correlation between hypokalemic alkalosis, hyperuricemia, and tubulointerstitial disease. Similarly, in patient #5 (H.B.), a variety of glomerular changes along with severe arterial vascular changes characterized the progressive nature of congenital nephrotic syndrome. The baby died 1 year after first evaluation.

An important role of EM in the study of membranous glomerulonephritis has been reported. This pathological condition is intriguing to the practicing physician

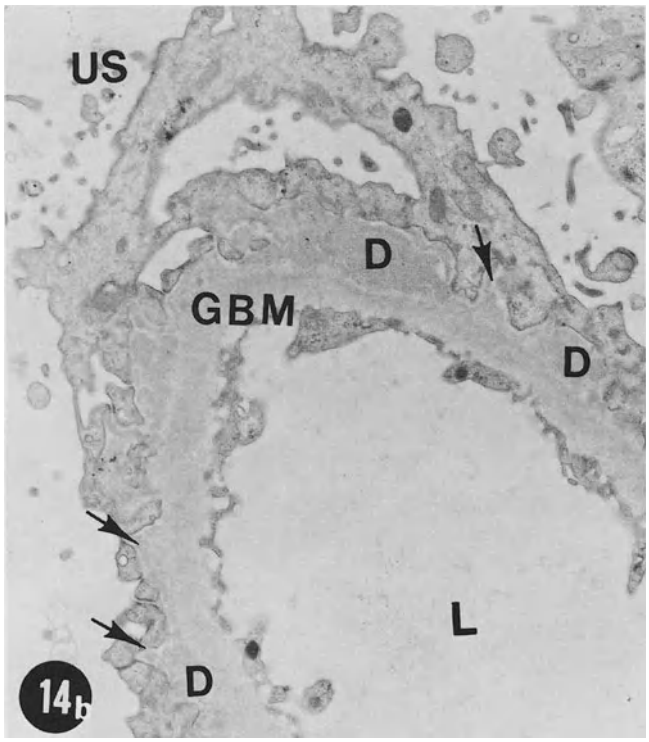

Fig. 12-14. *(Continued)*

because it is the most common cause of nephrotic syndrome in adults, and the therapeutic benefits from the use of corticosteroid or immunosuppressive therapy are still dubious. During the past few years, EM has made possible the staging of membranous glomerulonephritis (stages 1–4) according to an arbitrary number of spikes, as well as number, size, and electron density of the deposits. This type of histological assessment has potential value in deciding whether or not to institute corticosteroid therapy in this pathological process. There is good evidence to indicate that stage 1 or stage 2 membranous glomerulonephritis would respond to corticosteroid therapy. This is consistent with the author's own experience. Thus, patients with stage 2 membranous glomerulonephritis (illustrated in Figs. 6-30 and 6-31) underwent complete remission. It seems logical to consider that prospective studies of the therapeutic trial in all four stages of membranous glomerulonephritis and assessment of the results by serial biopsies (every 1–2 years) would add a great deal to the understanding of the natural history of membranous GN and the benefit of corticosteroid therapy in this disease.

EM has also been found to be a useful tool in determining the activity of the disease process. In lupus nephritis, for example, the pathological process is judged active by noting the presence and the amount of subendothelial deposits. Although subendothelial deposits generally disappear with treatment of lupus nephritis, the observations of other workers, along with our experience, do not support the concept that the disappearance of deposits denotes a cure of the disease. EM studies of serial renal biopsies from patient #6 (S.P.) are supportive of this notion. Although in her second biopsy no subendothelial deposit was found, the presence of a few epimembranous deposits and a few spikes in the GBM suggested membranous transformation of the initial proliferative GN. This histologic transformation predicted heavy proteinuria in the patient and the augury was substantiated by increasing proteinuria in serial 24-hr urine studies. For more details concerning assessment of activity of the pathological process, see Chapter 6.

Among other relative benefits derived from the use of this technique is its enormous resolution, which can reveal the anatomic pathology and pathogenetic mechanism in even a few viable capillaries or in a partly sclerotic glomerulus. This is especially important when the portion of the specimen fixed for LM study is inadequate or unsuitable (less than five glomeruli or no glomeruli, respectively).

Doubtful or Controversial Value

Although the yield of EM in diffuse proliferative glomerulonephritis is not nearly as high as it is in nephrotic syndrome and focal glomerulonephritis, disparities still exist between LM and EM evaluations in diffuse proliferative GN. In general, EM study does not add much information over and above that obtained from the LM study. This analysis, however, confirms the statement concerning diffuse proliferative GN made by my colleagues and me a decade ago.

It has been suggested that the presence of necrosis, thrombosis, or crescent

formation in acute proliferative glomerulonephritis implies a bad prognosis. The data of the present study fail to support this as a general statement. Similarly, the data of the present study do not comply with the apparent ominous significance of an excessive number of polymorphonuclear leukocytes (PMN) within the glomerular capillaries in glomerulonephritis. We have observed segmental necrosis of the glomeruli, excessive exudation of PMN into the glomerular capillaries, and thrombosis in the peritubular capillaries in poststreptococcal glomerulonephritis (Figs. 12-15 and 12-16; see also Figs. 6-1, 6-9 to 6-12). Despite these severe histological changes, both patients have shown complete clinical recovery. Follow-up studies have suggested no evidence of residual disease.

Several reports have discussed typical "humps" as an index to assess the activity of the pathological process and to predict clinical recovery. Churg and Grishman (1975) have stated that "typical" and "atypical" humps indicate good and bad prognoses, respectively. The clinical and histological studies of patients reported herein both support and contradict these authors' views. In patient #7 (E.G.) there were no typical "humps" observed; deposits were mainly intramem-

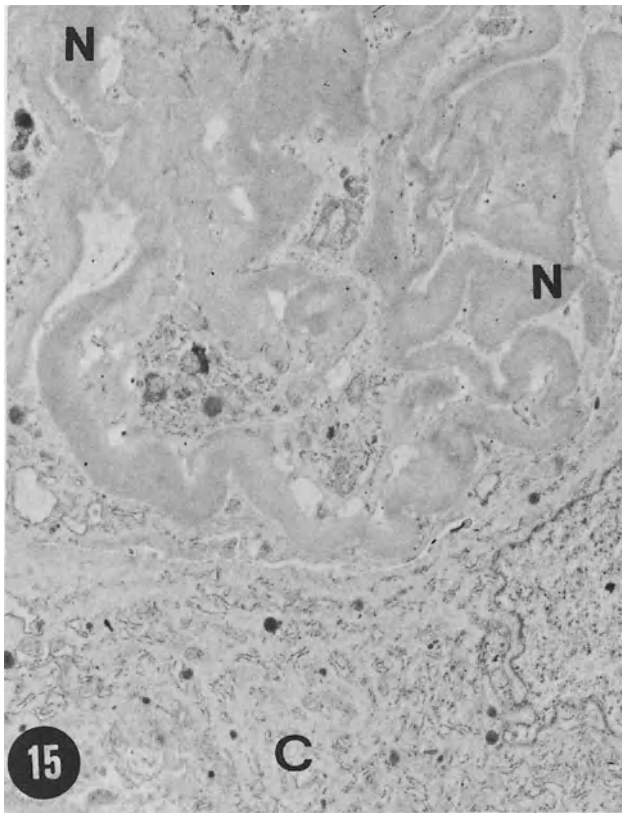

Fig. 12-15. Complete necrosis (N) of capillary loops of the glomerulus which also demonstrated typical "humps," as shown in Fig. 12-16. A part of the crescent (C) is seen (UA + LC, ×3400).

branous and the proliferation was mild in the first biopsy (Fig. 12-6). The patient remained asymptomatic for about a year at which time she had a recurrence of symptoms with greater severity and a worsening of the renal function, but the EM of the kidney obtained during this episode demonstrated numerous typical "humps" (Fig. 12-8). After this episode, she made an uneventful recovery. Follow-up clinical studies 2 years after the second biopsy revealed no evidence of residual disease. The relationship between the two episodes is difficult to determine. Whether these two episodes were separate or the second episode was an exacerbation of the first remains elusive. The finding of typical "humps" in the second biopsy and the clinical recovery following the second episode constitute conceptual evidence that typical "humps" signify a better prognosis. This view is contradicted by the finding of typical "humps" in patient #3 (F.W.) who presented with nephrotic syndrome which did not undergo remission. Other authors have reported similar observations. This type of adverse relationship between the histologic findings and the clinical course justifies regular use of EM to facilitate improvement of our knowledge in diffuse proliferative glomerulonephritis.

SUMMARY

1. The values of EM studies of renal biopsies from patients with glomerular and tubulointerstitial diseases have been assessed as absolute, relative, and controversial.

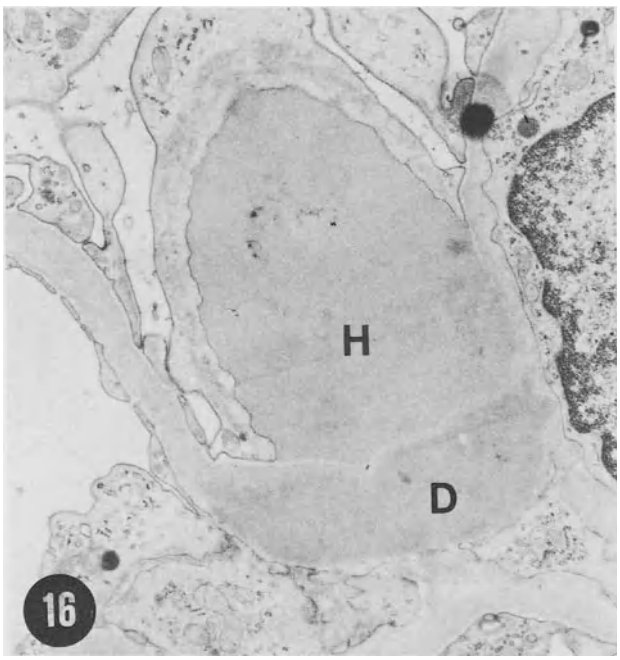

Fig. 12-16. A typical "hump" (H) characterizes poststreptococcal glomerulonephritis. The adjacent segment of the glomerular basement membrane also demonstrates deposit (D) (UA + LC, ×6200).

2. EM has been found to be absolutely valuable in nephrotic syndrome. It can distinguish lipoid nephrosis from stage 1 membranous GN which may appear normal by LM. In this respect LM is deceptive to the practicing physician.

3. EM appears to be absolutely valuable in the evaluation of focal glomerulonephritis, asymptomatic proteinuria, and recurrent hematuria.

4. EM has not been found superior to LM in the anatomical diagnosis of diffuse proliferative GN. However, careful studies of "humps" may help distinguish poststreptococcal GN from other diffuse proliferative GN.

5. EM distinctly contributes in the assessment of activity of the pathological process. This has been of great value in the management of lupus GN.

6. Accurate staging of membranous glomerulonephritis can be achieved by EM. This type of study is the key to institute therapy in membranous GN and in understanding the natural history of this disease.

7. By virtue of its high power of resolution, EM has helped to make a proper diagnosis even in half of a glomerulus, when LM study has been inadequate (less than five glomeruli) or unsuitable (no glomeruli).

PART 2
STUDIES OF ARTERIAL VESSELS IN ESSENTIAL HYPERTENSION AND GLOMERULAR DISEASES

ESSENTIAL HYPERTENSION

No convincing need has yet been found to warrant routine renal biopsy study in essential hypertension. There are manifold reasons which deter implementation of this procedure as a routine practice in the care of patients with essential hypertension: (1) slight or no evidence of involvement of renal parenchyma in most, slow but progressive impairment of renal function in some, and rapid development of uremia in a few; (2) apprehension of severe postbiopsy bleeding; and (3) lack of delineation of the importance of tissue diagnosis in the overall evaluation and management of hypertensive patients.

A limited number of investigators, however, do perform renal biopsies in order to eliminate the possibility of renal parenchymal disease primary to hypertension. Consecutive studies of the renal tissues in a small number of patients have been made by a few investigators to evaluate the relationship between the morphological changes in the kidney and essential hypertension. We have had the opportunity to study renal tissues consecutively in several patients with severe or malignant hypertension and to assess the value of EM study of renal tissues in hypertensive patients. In this connection the clinical profile of a patient studied for 43 months has been described to exemplify the value of EM study of renal tissues in hypertensive patients (see patient #5, Chapter 9).

The value of EM study of renal biopsies in hypertension can be summarized as follows:

1. EM has proven to be an important technique in demonstrating specific renal arteriolar changes which characterize potentially severe (or malignant) hypertension (Fig. 9-9) when clinical manifestations are mild or absent. Therefore, this type of evaluation can predict the possibilities of progressive histological damage and worsening clinical hypertension.
2. EM can distinguish nonspecific arteriolar and glomerular changes in association with benign essential hypertension from the specific renal lesions seen in malignant hypertension (for details see Table 9-4).
3. EM has proven to be a useful technique for distinct evaluation of necrotic change, the presence or absence of fibrin, detailed changes in the glomerular and arteriolar basement membranes, changes in smooth muscle cells (SMC) and elastic tissue, and the presence or absence of fibroblasts and other infiltrative cells.
4. Arteriolar hyaline thickening by LM has been considered a characteristic feature of arteriolar (i.e., benign) nephrosclerosis. The hyaline material is homogeneous, eosinophilic, and moderately PAS positive. This LM finding may appear by EM as granular deposits, thickened basement membrane, and/or excessive BM-like materials (see Fig. 9-2 versus Figs. 9-3 and 9-4). Fisher and colleagues (1966) have suggested that the term hyaline is inappropriate.
5. The LM findings of an eosinophilic, intensely PAS-positive, and homogeneous dark material on trichrome staining (fibrinoid necrosis) in the arterial vessel have been considered by many as a hallmark of malignant hypertension. The term fibrinoid necrosis is inaccurate since fibrin is seldom found in the arterial vessels of the kidney in hypertensive patients or in spontaneously hypertensive rat (see Figs. 9-22 and 9-30). Diffuse necrosis of the smooth muscle cells (Figs. 9-16 and 9-30) apparently provides the light microscopic appearance of intensely PAS- and trichrome-positive material.
6. EM distinctly demonstrates hyperplasia and hypertrophy of SMC (see Figs. 9-14, 9-15, and 9-22). Proliferated SMC forms bundles; hyperplastic SMC is characterized by single large or notched nucleus or multiple nuclei, prominent attachment plates, many rough-surfaced endoplasmic reticula and numerous ribosomes.
7. Infiltration of the arteriole by a variety of cells such as fibroblast, platelet, lymphocyte, and neutrophil can be confirmed by EM study only. Misinterpretation of inner SMC as fibroblasts and inflammatory cells by LM can be avoided when the specimen is further studied by EM.

ULTRASTRUCTURAL ANALYSIS OF RENAL ARTERIOLOPATHIES IN GLOMERULAR AND TUBULOINTERSTITIAL DISEASES

During EM study of glomeruli, we observed abnormal arterial vessels in focal glomerular sclerosis and congenital nephrotic syndrome. We extended the

observations and were able to study arterial vessels in the renal biopsies of 32 patients from a total of 143 patients (22%) with a variety of glomerular and tubulointerstitial diseases. The vessels comprising mostly large and small arterioles and rarely small arteries were found in the thick sections which were prepared primarily for the studies of glomeruli in the biopsy specimens. These arterial vessels were studied separately or along with glomeruli. The analysis has revealed that with some exception in lipoid nephrosis all types of glomerular and tubulointerstitial diseases are associated with abnormal vessels. Of the types of vessels, large arterioles are almost always found to be pathological. Small arteries, although rarely found, reveal similar or even more severe abnormalities than those in the large arterioles. Small arterioles and peritubular capillaries are found to be abnormal less frequently and to a lesser degree. In general, the vascular abnormalities comprise variably thickened basement membranes between endothelial cells and smooth muscle cells and between individual smooth muscle cells, atrophic and/or necrotic smooth muscle cells, electron-dense deposits, cystic changes, and mild to marked increases in collagen fibers and elastic tissue (Figs. 12-17 to 12-20).

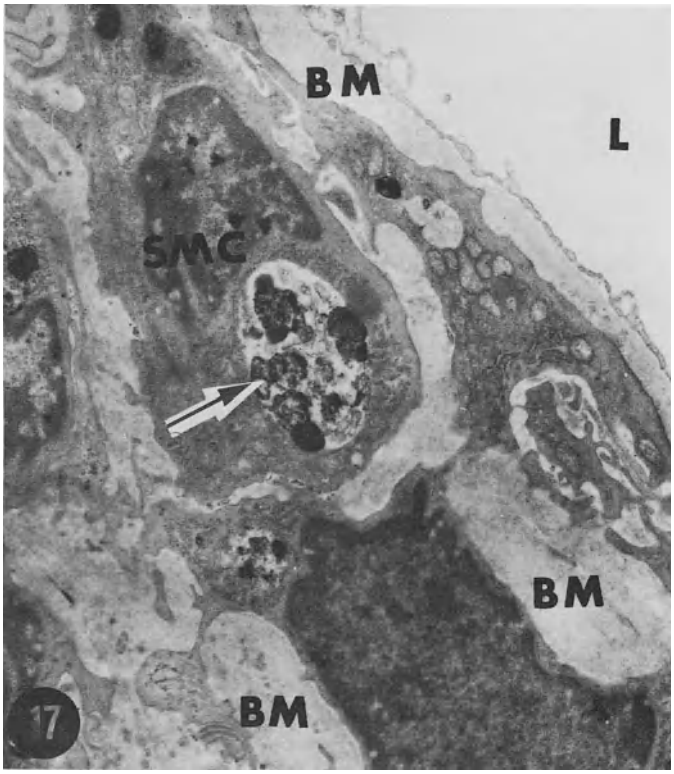

Fig. 12-17. A large arteriole demonstrates thickening and thinning of the basement membrane (BM) between endothelial cell and smooth muscle cells (SMC), thickening of BM between individual SMC, excessive basement membrane-like material formation, and atrophy of SMC. Lysosome in SMC (arrow) and lumen of the arteriole (L) are shown (UA + LC, ×15,000). From a patient with focal glomerular sclerosis. The glomerular changes from the same biopsy are shown in Figs. 6-52 to 6-54.

The mechanisms of vascular lesions in glomerular and tubulointerstitial diseases are unclear. The vascular changes described herein, such as combinations of sclerosis (thickened and irregular basement membranes) and atrophy of smooth muscle cells, necrosis of smooth muscle cells, electron-dense deposits, and increased amounts of elastic tissue and collagen fibers, have been observed in the renal arterioles in essential benign and malignant hypertensive patients, respectively. This similarity raises a question as to whether or not the aforesaid vascular lesions are caused by hypertension which is often associated with glomerular and tubulointerstitial diseases. This is unlikely in our analysis, since 15% of the

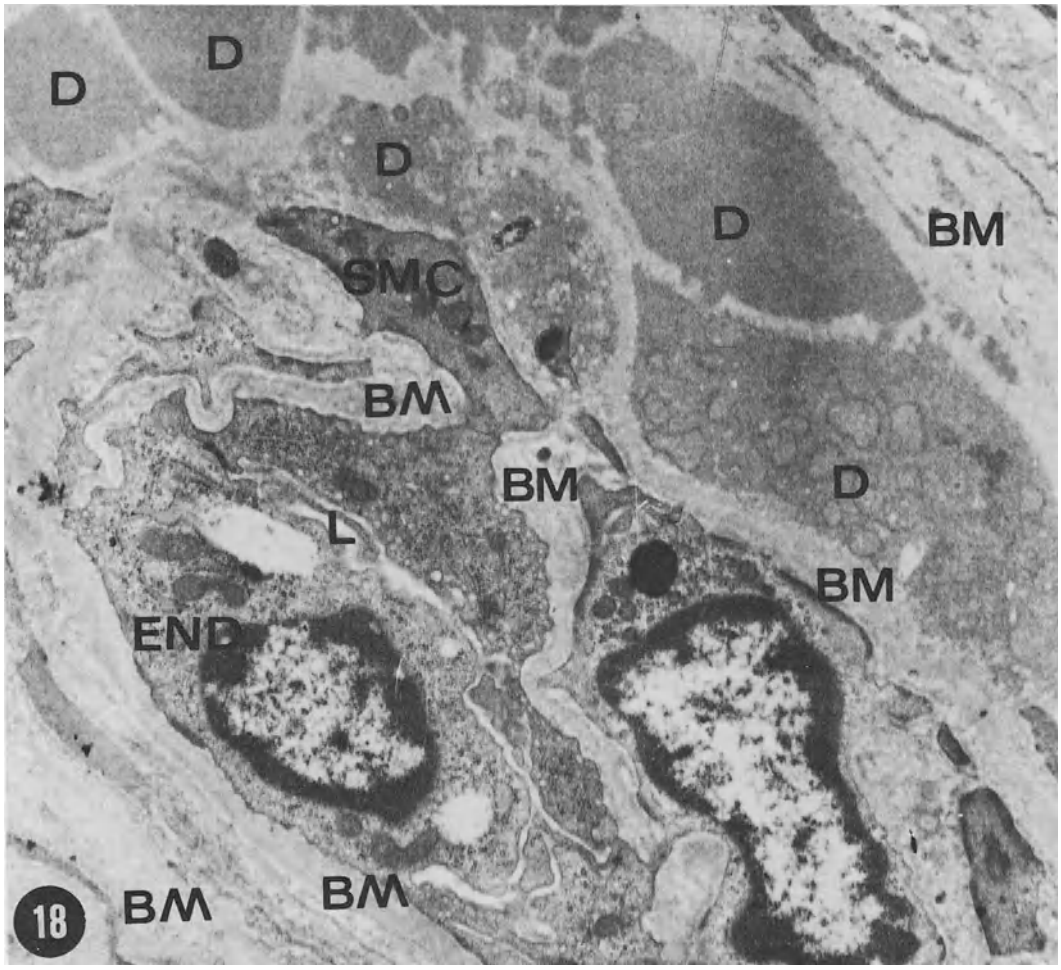

Fig. 12-18. A large arteriole shows thickened basement membrane (BM) between endothelial cells (END) and smooth muscle cells (SMC), marked thickening of the basement membrane between individual SMC, and atrophy of SMC. Note pronounced electron-dense deposits (D) in the SMC layer. From a patient with focal glomerular sclerosis, in whom EM of glomeruli demonstrated massive electron-dense deposits (Fig. 6-55) (UA + LC, ×18,000).

patients were hypertensive. Furthermore, no difference was observed in the type or severity of vascular lesions between the patients with hypertension and those without it.

The ultrastructural features in the vessels resemble those in the glomeruli and tubules. Thus, all three component parts of the kidney, i.e., glomeruli, tubules, and arterioles, demonstrated thickened BM in focal glomerular sclerosis and hereditary nephritis (Fig. 12-17); electron-dense deposits in poststreptococcal

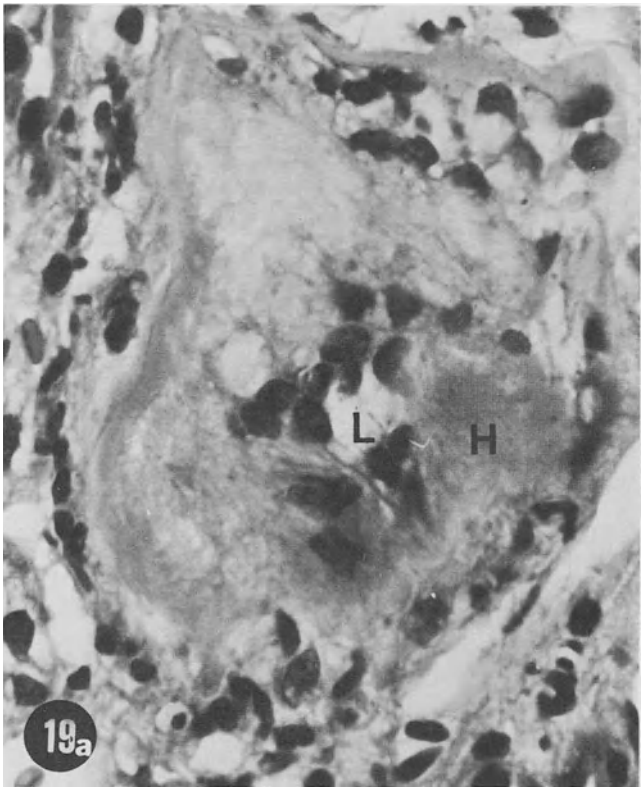

Fig. 12-19. (a) A large arteriole shows normal endothelial cells but replacement of smooth muscle cells by homogeneous eosinophilic and PAS-positive materials. Conspicuous collection of this type of material (H) has been variously described as hyaline material, hyalinosis, and so on. Lumen (L) of the arteriole is compromised (PAS, ×400). (b) This large arteriole shows thickening of the basement membrane (BM) between endothelial cell (END) and smooth muscle cells (SMC) and between individual SMC. Note segmental expansion of the BM by large electron-dense deposits (D). The deposits are of poor electron density, like those in the glomeruli (Fig. 6-33). The SMC are atrophic. The arteriolar changes are not consistent with renal lesions of hypertension (for more details see Chapter 9) (UA + LC, ×16,000). Parts (a) and (b) are from the renal biopsy of a patient with stage 3 membranous glomerulonephritis. (c) A large arteriole demonstrates thickened basement membrane (BM) between endothelial cell (END) and smooth muscle cells (SMC) and between individual smooth muscle cells. Note electron-dense deposits (circles) between the SMC. Lumen (L) of the arteriole is shown (UA + LC, ×20,000). From a patient with lupus proliferative glomerulonephritis. For EM of glomeruli see Figs. 6-13 to 6-15.

GN, lupus GN, idiopathic membranous GN, focal glomerular sclerosis, and transplant rejection (Figs. 12-18, 12-19b, 12-19c); cellular necrosis in lupus GN, mesangioproliferative GN, and rapidly progressive GN; and cystic changes in the congenital nephrotic syndrome (see Figs. 7-18 to 7-21). These similarities suggest that common mechanisms such as deposition of immune complexes may be operating in the pathogenesis of lesions among the glomeruli, tubules, and vessels (Fig. 12-21).

The relationships between the vascular lesions and the clinical profiles or renal functional status cannot be ascertained from the data in this study. In a report on irreversible renal failure in idiopathic nephrotic syndrome, Raij *et al.* (1976) have suggested that irreversible failure of renal function may be related to

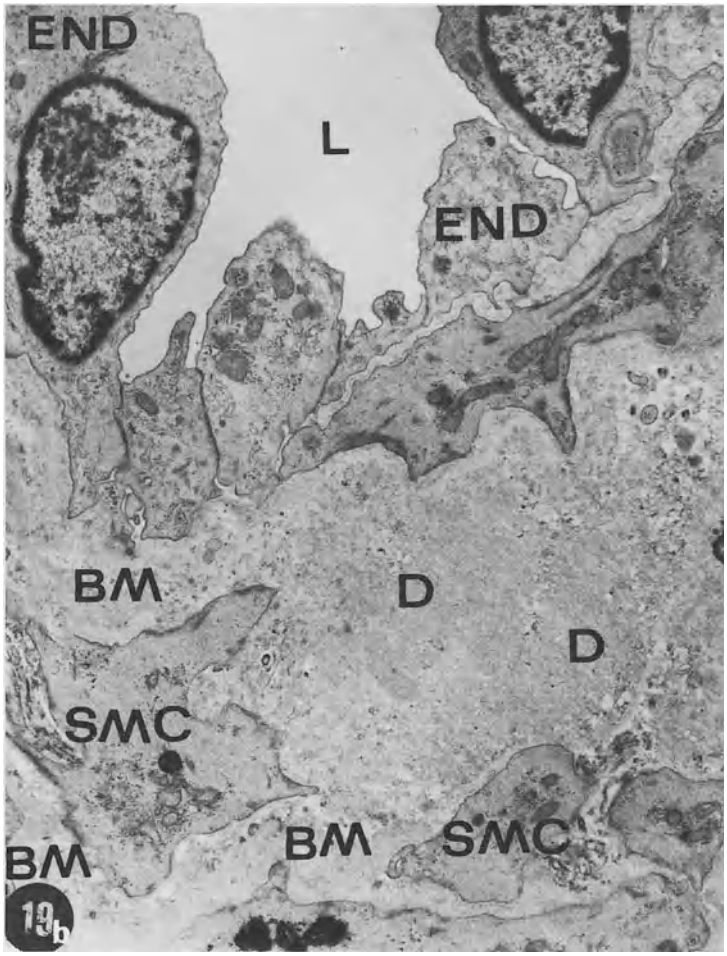

Fig. 12-19. *(Continued)*

permanent alterations of renal blood flow or ultrastructural changes, or both. The abnormal renal arterioles demonstrated by EM in this series could account for permanent and severe alterations of renal blood flow and thus contribute to renal failure in glomerular and tubulointerstitial diseases.

SUMMARY

1. EM study of small arterial vessels of the kidney in essential benign and malignant hypertension has demonstrated disparate pathological changes. This is

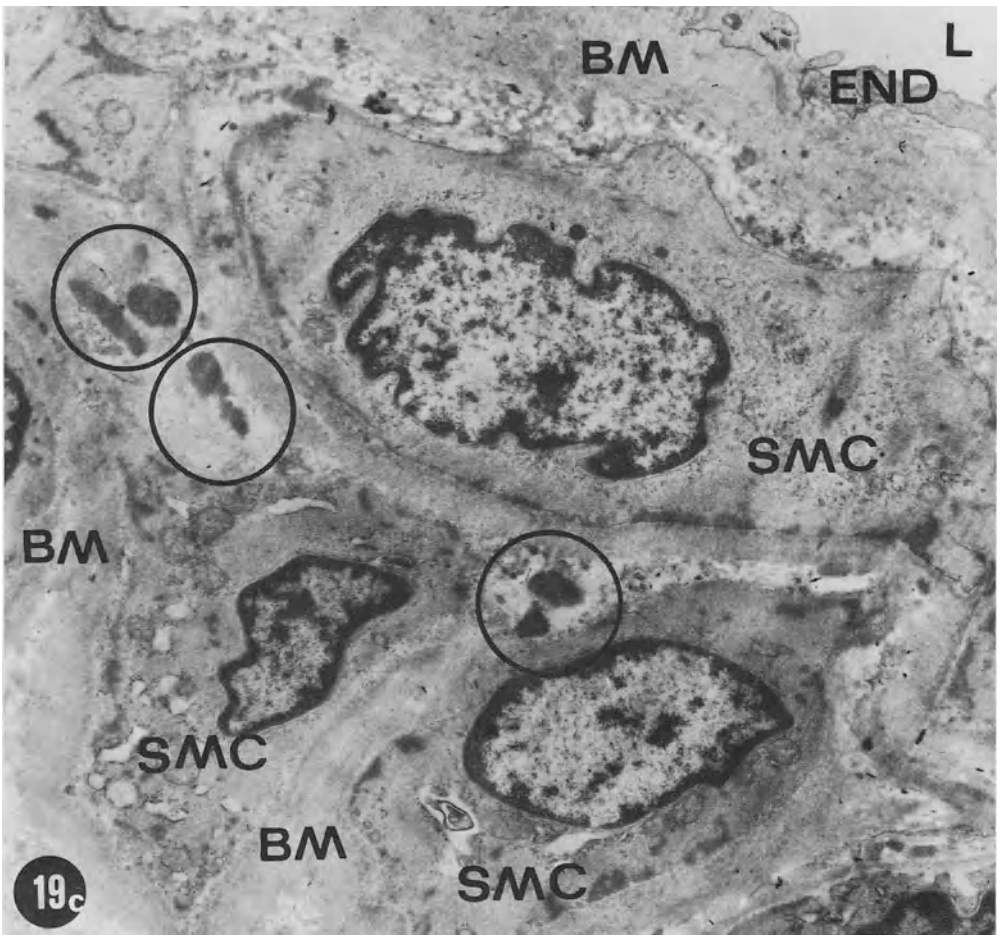

Fig. 12-19. *(Continued)*

often not recognizable by LM study. The renal pathology in benign hypertension appears to be nonspecific whereas the renal pathology in malignant hypertension is clearly related to hypertension. Because of disparate features, it is an unlikely possibility that the renal pathology in benign hypertension would ever progress to that observed in malignant hypertension. By virtue of delineation of potentially severe changes in the arterial vessels, EM study of renal biopsies can predict progression of the pathological process and evolution of clinically severe (malignant) hypertension in patients who initially present with less severe hypertension.

2. EM has shown definite promise in the investigation of vascular pathology in glomerular and tubulointerstitial diseases. Since the findings in the arterial vessels studied thus far resemble those in the glomeruli and tubules, it appears that arterial vessels share the brunt of lesions with glomeruli and tubules in a variety of disease processes. The clinical application of this observation is still poorly understood.

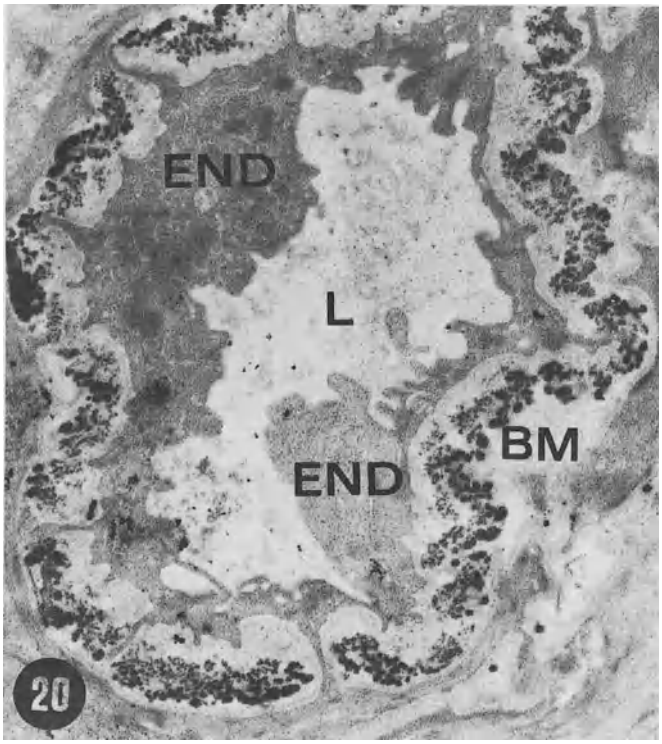

Fig. 12-20. A small arteriole exhibits excessive amounts of amorphous electron-dense elastic tissue within the basement membrane (BM). Lumen (L) and endothelial cell (END) are shown (STPPSX, ×7000). From a patient with poststreptococcal glomerulonephritis.

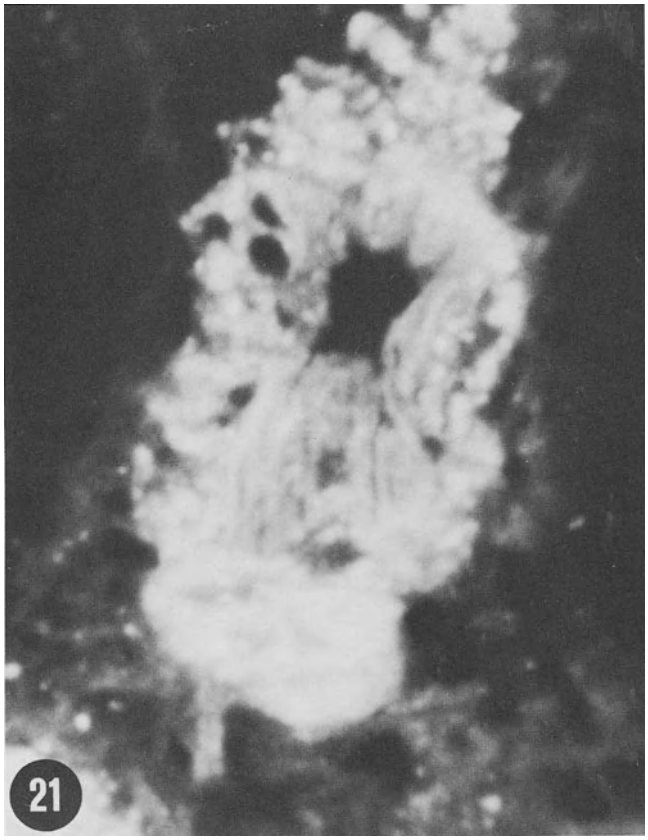

Fig. 12-21. A strongly positive (4+) fluorescence of IgG in a granular pattern throughout a large arteriole (IFM, ×1600). From a patient with lupus proliferative glomerulonephritis.

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13 Principles of Management Pertaining to Renal Pathology

James E. Wenzl, M.D.

INTRODUCTION

It is an established fact that no other branch of medicine has undergone as rapid or as astonishing an amount of growth as has the field of nephrology during the past two decades. In 1960, the field was characterized by a loose association of anatomists, physiologists, pathologists, and internists who were interested in the structure and function of the kidney in health and disease. They were soon joined by a small but growing group of immunologists and pediatricians who were chiefly concerned with renal immunopathogenic and pathophysiologic mechanisms. The main thrust of the specialty concerned itself with diagnosis of renal disease and treatment of the pathophysiologic consequences of the various forms of renal dysfunction.

During the 1960s, rapid advances in immunology, coupled with astonishing breakthroughs in gaining long-term vascular access and technological advances in the mechanics of extracorporeal dialysis, resulted in an exponential increase in both the number of physicians involved in this area and in the level and amount of care being given to renal patients. These patients, whose outlook had previously been considered to be gloomy at best, can now often look forward to months or years of reasonably productive life. At the time of this writing, at least 16,000 renal transplantations have been performed, and approximately 40,000 patients with end-stage renal disease are being cared for in the United States alone. The cost of treatment for these patients is currently estimated to be approximately one billion dollars and is subsidized mainly by the federal government. This phenomenal growth in the technology and availability of renal dialysis is both a cause and a result of the present treatment situation.

The current national emphasis on treatment of end-stage renal disease is at best a palliative one, and all observers agree that a more logical and efficient allocation of medical and financial resources would have been to the prevention and treatment of renal disease before end-stage renal failure has occurred. During the past two decades, clinical and investigational trials of various forms of therapy have been reported, but both the number and results of such trials have been disappointing, particularly when contrasted with the efforts and resources allocated to the treatment of end-stage renal failure. The current federal emphasis on direct patient treatment and deemphasis of support of investigational efforts will probably continue this unfortunate trend for the foreseeable future. This trend is especially regrettable inasmuch as transplantation therapy, at least for the foreseeable future, would appear to be more palliative than curative, and carries mortality and morbidity risks which are higher than those which would be expected if satisfactory forms of specific drug therapy were available for most of the conventional forms of renal disease.

During the past two decades, an impressive array of pharmacological preparations have been tested in both the laboratory and clinical investigational setting as possible therapeutic agents for arrest or eradication of most forms of renal disease. Among the various agents tested are the corticosteroids, azathioprine, chlorambucil, 6-mercaptopurine, nitrogen mustard, cyclophosphamide, indomethacin, cyproheptadine, heparin, dipyridamole, aspirin, and ascorbic acid. In only occasional and usually well-defined disease entities has any consistent or expected benefit been shown to have occurred following treatment with those agents. The clinical literature abounds with uncontrolled observations in both children and adults of apparent beneficial effects from one or another of such forms of therapy which are later not borne out by more prolonged follow-up or by carefully controlled clinical trials. To further complicate this unfortunate situation, the majority of trials, including those controlled, have not been done on patients on whom complete renal tissue examination was conducted preceding and following initiation of therapy. Many of these trials have not been based on sound preexisting pathophysiologic evidence which would indicate the likelihood of a good result. In certain studies, it has been apparent that there has been acceleration of renal disease following discontinuation of apparently ineffective therapy as well as an increased morbidity and mortality rate due to complications of the drugs chosen for study. This is especially regrettable when the drugs are not favorably affecting the outcome of the disease process of at least a portion of the patients chosen for such therapy.

In terms of perspective, it is quite clear that there is no justification for exposing a child or an adult to the risks inherent in long-term administration of potentially toxic agents unless such treatment has previously been demonstrated to be beneficial for the clinical and histologic lesion being treated, or unless such agents are being administered as a part of a controlled clinical trial under the most vigorously controlled circumstances. Medicational treatment of renal disease is clearly one instance in the practice of medicine in which the more facile

choice is to opt for treatment, but the far more difficult course for the therapist (and more advantageous to his patient) is to resist the pressure to do something which is active but which may further complicate or deteriorate the patient's already declining health. It is patently illogical to continue to pursue such anecdotal trials without vigorous definition of the disease being treated, including baseline and follow-up histopathologic study of the renal lesions under treatment. In the context of experimental medicine, this must include complete study of the renal biopsy specimen, using light, immunofluorescence, and electron microscopy (EM).

With this background in mind, we will attempt to summarize the current indications for specific drug therapy, particularly as they are based on histopathologic definition of the underlying renal lesion.

THE NEPHROTIC SYNDROMES

Idiopathic Nephrotic Syndrome (Lipoid Nephrosis)

The only major renal disorder to date for which an expected and predictably favorable outcome from drug therapy may be uniformly anticipated is the idiopathic nephrotic syndrome (lipoid nephrosis, minimal lesion disease). Under ordinary circumstances, specific drug therapy has evolved only after a clear understanding of the pathophysiologic event causing the disorder has been elucidated, but the etiology and nature of the epithelial cell and foot process defect in this common renal illness remain elusive. Despite the lack of definition of the underlying mechanisms, a vast array of clinical experience has clearly demonstrated that corticosteroid therapy positively influences both the mortality and morbidity from this disorder. In view of the high mortality and morbidity rate of the untreated disease condition, the side effects of such therapy become unimportant considerations in judging therapeutic necessity. The general principles of treatment are summarized in Table 13-1. Differences in responsiveness to therapy between adults and children are summarized in Fig. 13-1.

In recent years, the major shortcomings of corticosteroid therapy, namely unacceptable side effects such as cataracts and dwarfism in children who have the relapsing form of the disease, have been shown to be indications for alternative forms of drug therapy, including the use of cyclophosphamide and chlor-

Table 13-1
Lipoid Nephrosis: General Principles of Treatment

1. A firm diagnosis based on renal histopathology should be established.
2. Treatment should be started as early as possible after accurate diagnosis.
3. Initial therapy should be continued at least 6–8 weeks to determine the type of response.
4. The patient and the family should be prepared for continued studies for at least 5–10 years.
5. Relapses should be treated with multiple courses.

ambucil. Both of these latter drugs have been shown to be extremely effective agents for treatment of the relapsing form of the disease (steroid dependency) in which clinical side effects have made further treatment with steroids unsatisfactory. Both of these drugs have potentially serious toxic side effects, however, including possible oncogenic as well as gonadal injury potential, so that their use must be carefully monitored in the clinical setting. In children especially, it is common practice in some centers to institute steroid therapy for the nephrotic syndrome without a pretreatment renal biopsy study. This practice is acceptable provided the patient demonstrates a rapid and apparently complete response, but if the response is incomplete or if early and frequent relapses occur, it remains a necessity to seek histopathologic definition of the underlying renal lesion before institution of therapy with either cyclophosphamide or chlorambucil since their clinical toxicity is considerable, the effects of these drugs on the growing child are not completely defined, and the predictability of response of the nephrotic syndrome in those lesions other than minimal lesion disease is uncertain. Despite these present vagaries and despite the fact that successful treatment remains empirical inasmuch as we are not certain of the nature of the favorable response, this well-defined renal histopathologic entity serves as a model for successful renal drug therapy and may eventually be used in treatment of other potentially serious or fatal forms of renal disease.

The other histologic lesions which may mimic the idiopathic nephrotic syndrome, many of which may give an identical clinical picture, including focal glomerular sclerosis, mesangial proliferative glomerulonephritis, and so on, are uncommonly and unpredictably responsive to either steroids or other immunosuppressive therapy. The benefits of such therapy must be carefully weighed against the side effects and probable lack of responsiveness in such patients, and must be left to the discretion of the individual nephrologists caring for such patients. Certainly, immunosuppressive therapy should be discontinued if an apparent beneficial effect is not achieved within a period of 6 to 8 weeks. Those patients with focal glomerular sclerosis pose an especially vexing problem, inasmuch as this lesion may easily be missed on biopsies taken early in the disease, especially if tissue from the corticomedullary region of the kidney is not present in the biopsy specimen. The presence of such a lesion should be suspected in

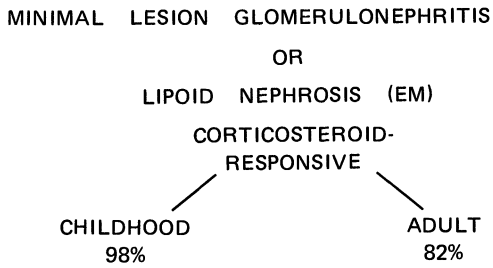

Fig. 13-1. Corticosteroid responsiveness in childhood and adult nephrotic syndrome due to lipoid nephrosis.

children who appear to have the idiopathic nephrotic syndrome both clinically and histologically, but who remain unresponsive to conventional steroid or immunosuppressive therapy.

Membranous Glomerulonephritis (Membranous Glomerulopathy)

The course of membranous glomerulonephritis is ordinarily an indolent and slowly progressive one, characterized by either slow progression to renal failure or spontaneous remission(s). Remissions in the adult patient, although not uncommon, do not necessarily correlate with a good outlook; Cameron (1972) has noted that only 25% of affected patients will be alive and without renal failure 10 years after the initial diagnosis. In children, the disease occurs less frequently, but appears to have a somewhat higher spontaneous remission and recovery rate. Well-documented cases of spontaneous clinical and histologic remission have been noted; we have observed such as instance in a 3-year-old child at our institution.

We have studied the effects of combined corticosteroid and azathioprine therapy in 13 adult patients (aged 22–64 years). During the follow-up period (average of 51 months), three progressed to renal failure, three remained stable, and seven had a significant decrease in proteinuria and stable or increased inulin clearances. In those five in whom EM study of serial biopsies was performed, there was no progression of staging of the membranous glomerulonephritis. However, because of the often indolent nature of the disease and the tendency for spontaneous remissions, coupled with the usual slow progression of the disease, it is very difficult to draw any firm conclusions from our study or from the other clinical studies that have been reported to date. The use of steroid treatment of this condition remains uncertain; the risks of such therapy must be balanced against unpredictable benefits (if any). The use of immunosuppressive drugs is even more dubious.

Documentation of the pure membranous lesion is essential, so that a rational approach to therapy may be planned. Electron microscopy is a requisite for absolute delineation of the entity. Following establishment of the entity, it is the author's personal opinion that drug therapy, if offered, should be given on the basis of a frank and open discussion with the patient about its uncertain results and possible side effects. If it is the combined feeling of the clinician and the patient that such therapy is desirable, then it should be undertaken and continued in relatively high dosage for a long period of time or until remission or lack of response is evident. The decision to withhold steroid or immunosuppressive therapy for individual patients also appears to be a tenable option.

Mesangiocapillary Glomerulonephritis (Membranoproliferative Glomerulonephritis)

As in the case with membranous glomerulonephritis, the course and natural history of this disease are not easily predictable for the individual patients. In

general, renal failure at the onset of the disease, the presence of a persistent nephrotic syndrome, or the histologic identification of extracapillary proliferation indicates a poor prognosis. As in the case of membranous glomerulonephritis, this disease appears to have a somewhat better prognosis in children than in adults. Further studies of the therapeutic efficacy of steroids and immunosuppressive agents appear warranted, especially in view of the gloomy prognosis of the unmodified disease in the adult. Future clinical trials must be controlled and include careful attention to electron microscopic definition of the types of membranoproliferative glomerulonephritis (type 1, 2, and 3) present. In a small group of adult patients treated with multiple therapeutic agents, Kincaid-Smith (1973) found an unusually high 3-year survival of 82%, although her data utilized a retrospective survey of untreated patients. McAdams and co-workers (1975) have found an apparent benefit from continuous low-dose prednisone for children afflicted with this disorder. A long-term, multicenter trial with alternate-day steroid therapy would seem warranted, but it needs to be based on careful electron microscopic definition of the individual types of membranoproliferative glomerulonephritis under treatment.

Proliferative Glomerular Lesions Associated with the Nephrotic Syndrome

These include focal proliferative glomerulonephritis, diffuse endocapillary proliferative, and endocapillary and extracapillary proliferative glomerulonephritis. Definition of these lesions by light and electron microscopy generally identifies a group of nephrotic patients who are unresponsive to steroid or other drug therapy. The risks of such therapy for these patients appear to far outweigh any therapeutic benefits, and drug therapy is unwarranted.

Nephrotic Syndrome Associated with Underlying Diseases

The various entities included here have already been discussed in Chapter 7. In general, specific drug therapy has little to offer for any of these patients other than those with systemic lupus erythematosus and possibly rapidly progressive glomerulonephritis. The nephrotic syndrome may sometimes be controlled by withdrawal of the specific offending toxin or allergen, or treatment of an underlying infectious or neoplastic disease. In general, therapy of the primary condition is the treatment of choice.

GLOMERULONEPHRITIS

Lupus Nephritis

The various patterns of histological involvement of the kidney in systemic lupus erythematosus have already been described in the chapter on anatomical

pathology of glomerulonephritis. In general, those lupus patients with focal and segmental proliferative glomerular lesions (mild lupus proliferative glomerulonephritis) have a good prognosis and tend to remain responsive to drug therapy. Renal failure is uncommon if the lesion remains focal on rebiopsy 2 to 4 years after onset. The membranous lesions and the diffuse proliferative glomerular lesions in lupus nephritis are commonly associated with a nephrotic syndrome. The latter is also associated with a tendency to progress to renal failure; if there is extensive extracapillary proliferation, this progression may be very rapid. The presence of subendothelial and mesangial electron-dense deposits on electron microscopy often correlates with severe diffuse proliferative disease and a tendency toward rapid onset of renal failure. The finding of electron-dense deposits in the intramembranous as well as in the subepithelial location correlates with membranous changes and does not in itself dictate an urgency for immediate, aggressive therapy to prevent rapidly progressive renal disease.

The scope of therapy must be determined on the basis of histologic changes and clinical expression of the disease. In general, patients with mild clinical renal disease and segmental proliferative changes on electron microscopy are not at high risk to develop renal failure. In these patients, extrarenal manifestations of systemic lupus erythematosus, or injudicious, overaggressive therapy are more likely to result in death. Infection from fastidious organisms which may invade the patient because of the underlying disease and overly aggressive therapy pose a major threat to life. However, it is necessary to ascertain on rebiopsy that an initially focal proliferative lesion is remaining stable; we have seen progression of renal disease to renal failure within 2 years after an initial biopsy which showed an apparent focal proliferative nephritis. Electron microscopic demonstration of subendothelial deposits in any number in an otherwise focal proliferative lesion indicates a greater likelihood of progressive disease, and hence a greater need for aggressive therapy with steroids and/or immunosuppressive agents. In lupus patients displaying membranous changes, the prognosis for development of renal failure is roughly similar to that of patients with idiopathic membranous glomerulonephritis. In the case of lupus patients, however, therapy with steroids is definitely indicated inasmuch as these drugs are useful in controlling the extrarenal manifestations of the disease, and also appear to exert a beneficial effect on the course of the membranous changes in the kidney.

The situation for patients presenting with diffuse proliferative lesions, especially if complicated by azotemia at or near the onset, is distinctly different. These patients tend to have a very poor prognosis in terms of rapid onset of renal failure, and in general are less responsive to drug therapy. Aggressive therapy is indicated. High-dosage steroids (60 mg/day) combined with either cyclophosphamide (2.5 mg/kg) and/or azathioprine (3 mg/kg) therapy are indicated as soon as the extent of the disease can be defined histologically. Although this author is aware of no long-term well-controlled studies demonstrating an absolute benefit from the use of steroids plus cytotoxic or immunosuppressive agents, a number of uncontrolled studies, plus personal experience, indicates that a long-term trial of these agents for patients so afflicted offers some hope of benefit. Recent

evidence has indicated that intravenous pulse therapy with methylprednisolone may be of benefit in patients with overwhelming immune complex deposition (0.5–1.0 g/day). In patients with severe clinical manifestations who demonstrate subendothelial dense deposits, such therapy may be tried.

The superiority of either cyclophosphamide or azathioprine as an additional agent in the treatment of lupus patients receiving steroids has not been demonstrated. Published clinical experience of short-term trials, plus its effect on T lymphocytes, suggests that cyclophosphamide may be the superior drug in terms of disease suppression. However, the toxic side effects, including sterility, alopecia and so on, present significant obstacles to its long-term use, particularly in the female of child-bearing age. The psychological effects of such therapy can and often are devastating to the younger female. Trials with all three agents (prednisone, cyclophosphamide, azathioprine) would seem to deserve consideration.

Recent reports have indicated that for those patients with lupus in which the major disease expression is in the kidney, renal transplantation may be an effective form of therapy for end-stage renal disease. Therefore, if the major manifestation or major clinical expression of systemic lupus erythematosus is its effects upon the kidney, an extremely aggressive approach, including renal transplantation therapy, seems indicated. Long-term results of such treatment have not yet been reported.

Rapidly Progressive Glomerulonephritis

Rapidly progressive glomerulonephritis is a clinical term often used to describe patients who develop progressive renal insufficiency and uremia within 6 months after onset of their illness. The disease is characterized by a fulminant, progressively downhill course, often accompanied by oliguria or anuria and terminating in end-stage renal failure. This form of clinical nephritis is a syndrome including diseases of unknown etiology as well as several systemic diseases which may severely affect the kidney (Table 13-2). Patients so affected are usually young to middle-aged males, although young children and females of any age may be afflicted. The most striking feature on light microscopy is the occurrence

Table 13-2
Classification of Rapidly Progressive Glomerulonephritis

Idiopathic
Poststreptococcal glomerulonephritis
Goodpasture's syndrome
Bacterial endocarditis
Polyarteritis nodosa
Anaphylactoid purpura
Systemic lupus erythematosus
Essential mixed cryoimmunoglobulinemia

of extensive extracapillary proliferation (crescents) usually affecting 50% or more of the glomeruli. In severe cases, nearly all glomeruli will show crescents. Electron microscopy is generally nonspecific; the principal findings include electron-dense deposits or electron-lucent areas within the glomerular basement membrane, fibrin thrombi, and, in those patients with anti-glomerular basement membrane (GBM) antibody formation, gaps or segmental discontinuities along the glomerular basement membrane. Patients in which the underlying etiology is associated with systemic lupus erythematosus or poststreptococcal nephritis may also demonstrate subepithelial deposits or "humps," but these are uncommon. However, if "humps" are present, the prognosis for recovery is better, especially if the disease is poststreptococcal in origin. In this instance, no specific therapy may be necessary. In those patients with anti-GBM antibodies, the outlook for recovery is very poor, especially if over 70% of the glomeruli are affected with crescents. Unfortunately, treatment of these severely affected patients is generally disappointing. Steroids alone are generally ineffective. If one excludes patients with poststreptococcal glomerulonephritis, polyarteritis, and systemic lupus erythematosus, the beneficial effect from steroids and/or steroids plus cytotoxic agents is difficult to substantiate. Uncontrolled studies utilizing heparin alone or with multiple drugs, including those inhibiting platelet aggregation and cytotoxic agents have indicated that some possible benefit from such aggressive therapy may be seen. Again, the information to date does not allow a rational approach to therapy of the individual patient presenting with such a lesion. If heparin therapy is to be used, its potential risks must be weighed against benefits, and treatment started very early in the course of the disease. Such risks can only be undertaken in the light of extremely careful and thorough definition of the histologic lesion, and after ruling out systemic diseases for which more specific therapy may be available.

Goodpasture's Syndrome

This syndrome is often included under the heading of rapidly progressive glomerulonephritis. The essential elements for this syndrome include the presence of pulmonary hemorrhage, glomerulonephritis, and anti-GBM antibodies. The clinical course may be identical to that of rapidly progressive glomerulonephritis, except for the presence of recurrent and sometimes life-threatening pulmonary hemorrhages. There is an even more predominant male-to-female ratio than in other forms of rapidly progressive glomerulonephritis. The electron microscopic findings are similar to other forms of anti-GBM antibody-mediated rapidly progressive glomerulonephritis. Steroids appear to have a beneficial effect upon the pulmonary lesion but not on the renal lesion. Similarly, cytotoxic agents have not been found beneficial for the renal lesion. Heparin therapy appears to be contraindicated in the presence of active pulmonary hemorrhages, and probably plays a minor role in the treatment of this disorder. Plasmapheresis will reduce anti-GBM antibody levels and may improve the pulmonary and renal lesions; however, this form of therapy is not widely available. In occasional patients,

spontaneous improvement in renal function may occur after a period of time on peritoneal dialysis or hemodialysis. We have observed two such patients at our institution within the past 5 years. Recurrent disease in cadaveric allografts may occur, further complicating the treatment of this progressive disorder.

Anaphylactoid Purpura (Henoch–Schonlein)

Renal disease associated with the triad of purpuric skin lesions, gastrointestinal bleeding, and joint involvement is known as anaphylactoid purpura. Approximately one out of three children with this disease has clinical renal involvement. Generally, the nephritis is mild and self-limited. The presence of crescents and/or the nephrotic syndrome may indicate a rapidly downhill course; indeed this disorder may occasionally be included with the rapidly progressive glomerulonephritis (Table 13-2). Steroids, although of benefit for the extrarenal manifestations of the disease, do not favorably influence the course of the associated nephritis. As in other cases of rapidly progressive glomerulonephritis, a combination of immunosuppressive drugs and heparin therapy may be tried in those patients with a rapidly progressive course in which more than 50% of the glomeruli show crescents. The benefits of such therapy, however, have not been clearly documented.

SUMMARY

1. Specific medical therapy of renal disease, other than for pyelonephritis, remains empirical for most disorders. At the present time, in only two well-defined histopathological conditions can a favorable response to therapy be predicted (idiopathic nephrotic syndrome, systemic lupus erythematosus).

2. At the moment, more may be expected from a fundamental attack on the underlying pathophysiological and immunological aspects of glomerular diseases than from continued random testing of largely empirical drug regimens. Such empirical therapy, if given to individual patients, should be given on a rational, informed-consent basis as part of a collaborative effort to define efficacy in a predetermined, well-defined clinical and histopathological situation.

3. Truly informed consent will necessarily disqualify many patients from such trials. Without prospective histological and clinical definition, and similar periodic follow-up, such clinical experimentation should be discouraged.

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