CHAPTER 1

Renal Biopsy: An Historical Perspective

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The History of Renal Pathology

- Renal Pathology in the Prebiopsy Era
- History of Renal Biopsy (Surgical and Percutaneous)
- Nomenclature/Terminology
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It is hard for the present day clinical nephrologist or nephropathologist to conceive how renal pathology was understood prior to renal biopsy.\(^1\)

The history of percutaneous needle biopsy of the kidney has been written many times and need not be recounted here in all its details.\(^2\)–\(^6\) However, having been involved in the study of the small renal tissue specimens obtained by this procedure from its very beginning in the United States, I would like to give an account of certain aspects of this history that have not been adequately reported and discussed in the past. In this review I deal primarily with the first 10 years of use of this technique (i.e., the period that goes from the fall of 1952 to the spring of 1961 when the CIBA Foundation Symposium on Renal Biopsy was held in London).\(^7\)

In July 1952 I returned to the full-time staff of the Department of Pathology of the University of Illinois College of Medicine in Chicago after a period of almost 5 years at the U.S. Army Medical Nutrition Research Laboratory, also in Chicago. As director of the Experimental Pathology Section, most of my work there had been on the role of different nutritional factors on wound healing, but late in 1950 I was directed by the surgeon general of the army to conduct an experimental study on the use of dextran, a newly developed plasma expander, in hemorrhagic shock, with particular regard to renal function. This research project had a high priority because at the time in Korea there was a very high incidence of viral hepatitis in wounded American and South Korean soldiers treated with infusion of pooled plasma. This work had given me some knowledge of renal physiology and especially considerable hands-on experience in the actual performance of discrete renal function tests.\(^8\)\(^9\)

Sometime in the fall of 1952 the first percutaneous renal biopsies taken by Robert M. Kark and Robert C. Muehrcke were received in Surgical Pathology at the University of Illinois. Dr. Kark was then a professor and Dr. Muehrcke a resident in the Department of Medicine. I had known both of them for several years because Dr. Kark had been chief of the Clinical Division at the Army Medical Nutrition Laboratory, and Dr. Muehrcke had been one of my students at the medical school. For reasons that are still not entirely clear to me, the small renal biopsy specimens could not be handled by the Surgical Pathology Laboratory and were entrusted to me by Dr. Granville A. Bennett, then the chairman of the department. At the time I had no particular experience in renal pathology, but perhaps Dr. Bennett remembered that I had done some work having to do with renal function tests and that in previous years I had been associated with Dr. Kark in the same research laboratory. In assigning the renal biopsy to me for study and interpretation, Dr. Bennett had said, “Try to make sense of these small specimens,” and I sensed his skepticism that such small specimens could provide adequate or reliable information. I am sure he doubted whether renal biopsies of this type would have much of a future.

Thus, my introduction to renal pathology was fortuitous. At the time, certainly I did not appreciate the tremendous opportunity being given to me, that is, the resource for studying renal pathology during the life of the patient and from a completely new angle. For sure, nobody could have predicted then how much renal biopsy would contribute to the understanding of renal diseases and to the creation of a new specialty in medicine: nephrology.

The Prebiopsy Era
(or What We Knew in 1952)

Until about 1950 what was known about the pathology of renal diseases was based almost exclusively on postmortem studies. Unavoidably, autopsy findings were likely to be representative of more severe and more chronic stages of disease, whereas acute lesions were seen only in those relatively rare instances in which death occurred within a few days or weeks after onset of the disease. Milder lesions were either not recognized or were attributed to postmortem changes. Further, no precise clinicopathologic studies could be con-
ducted because, as a rule, renal functional studies and blood chemical determinations often were not done in the preterminal period. Even if they were, the results would be difficult to interpret because of the multitude of complicating factors during the last days of life. Etiology and pathogenesis are obviously more difficult to investigate in well-established or more chronic stages of disease. In brief, autopsy pathology provided a sorely inadequate approach to reconstruct the natural history and course of a particular renal disease.

In the early 1950s pathologists, to some extent, still relied on the classic work of Volhard and Fahr on Bright's disease published in 1914.10 In the United States the books of Addis and Oliver (1931),11 Bell (1946),12 Allen (1951),13 and McManus (1950),14 the latter based on the periodic acid-Schiff reaction, were the major source of information at that time. Heptinstall's Pathology of the Kidney,15 the first book in which renal biopsy studies (including immunopathology and electron microscopy) were given proper recognition, appeared only in 1966. For glomerular diseases (Bright's disease) the clinical classifications of Volhard and Fahr,10 and of Christian,16 Addis,17 and Ellis18 were used but were increasingly thought to be unsatisfactory. Actually, more comprehensive classifications had been formulated by Volhard and Fahr and by Addis. Unfortunately, these classifications were based on a combination of clinical, morphologic, and even etiologic features with unavoidable confusion. Addis had emphasized the importance of complete, quantitative urine analysis, and great strides had been made in the development and use of discrete renal function tests, but the differential diagnosis of renal diseases was still totally inadequate.19,20 Several different clinical syndromes (i.e., acute glomerulonephritis, the nephrotic syndrome, acute and chronic renal failure, asymptomatic proteinuria, asymptomatic hematuria) were clearly recognized, but it was not clear at all whether one or more etiologically or histopathologically different diseases were the cause of each of these syndromes. What was not realized at the time was that one renal disease can have many different histopathologic patterns and clinical presentations. By contrast, one clinica

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Surgical Biopsies

During the first half of the century several investigators had emphasized the desirability of studying renal tissue during life for diagnostic purposes. Perhaps Gwyn21 in 1923 was the first to suggest that, when indicated, a renal biopsy should be taken at the end of an abdominal surgical procedure, stating that "a kidney can always suffer the loss of a millimeter of substance." However, most reports on surgical renal biopsies of that period are based on only a few cases and usually dealt with tumors, cysts, nephrolithiasis, and other surgical diseases. An exception was the studies of Castleman and Smithwick,22 in which biopsies were taken during abdominal sympathectomy for hypertension. An important conclusion of these studies was that renal arteriolar sclerosis appeared to follow and to be the result, rather than the cause, of hypertension.

Percutaneous Needle Biopsies

The possibility that a percutaneous needle biopsy could be obtained from a kidney was first demonstrated by Ball23 in 1934 who biopsied only palpable renal tumors. Lindblom24 in 1946 used a needle and by injecting diodram was able to differentiate renal cysts from solid tumors. Cazal25 in France (1949) and Perez-Ara26 in Cuba (1950) used the needle for the diagnosis of renal tumors. The first to use needle biopsies for the diagnosis of medical diseases of the kidneys were Alwall27 in Sweden (1952), Iversen and Brun28 in Denmark (1951) (Fig. 1-1), and Perdo et al.29 in Cuba (1953).
Fig. 1-1. The Municipal Hospital in Copenhagen, Denmark, where percutaneous renal biopsy of the kidney was first performed.

The technique of Iversen and Brun was brought to the United States by Parrish and Howe in 1953. At about the same time, Kark and Muehrcke in 1952 introduced several important changes in Iversen and Brun's technique (Fig. 1-2). As a result, the procedure became safer and much more comfortable for the patient. Within a few years, many investigators in Europe and the United States reported on increasingly larger series of cases. Gradually, as most clinicians adopted the technique of Kark and Muehrcke, experience in taking the biopsy and in interpreting the findings increased; the percentage of specimens adequate for diagnosis also increased from about 50 to more than 90 percent while the number of complications decreased. These results were obtained with the "blind" technique. Today practically all biopsies are taken with one of the many different methods to visualize the kidney and the position of the needle. Ultrasound is the preferred method in most centers because it does not require radiographic material and the kidney can be localized even when renal function is severely compromised.

There does not seem to be much question that the desire to obtain biopsies for the diagnosis of medical diseases of the kidney was inspired by the clinically important contributions that had derived from widespread use of needle biopsies of the liver. Iversen and Roholm had been among the first to take needle biopsies of the liver on a large scale. The needle used by them (Roholm's cannula) was the same one later used by Iversen and Brun for the kidney. Although the kidneys are smaller, deeper, and less accessible than the liver, obtaining renal tissue by the percutaneous needle technique was considered feasible, as several clinicians and pathologists had had the experience that in performing needle biopsies of the liver, they unintentionally obtained renal rather than hepatic tissue (Pardo V, personal communication, 1994). Kark, who had been a student of liver and nutritional diseases before becoming involved with the kidneys, recounts that "like others I aimed at a patient's liver and pulled out renal tissue. No harm resulted." In one of the earliest series of percutaneous renal biopsies by a group of investigators from Dakar, French West Africa,
at the beginning of their paper the authors state "pratiquer sans le savoir une ponction du rein alors qu'on pense faire une ponction du foie" ("without realizing it take a biopsy of the kidney when meaning to take a biopsy of the liver"). No complications ensued.38

It is also pertinent to mention that at the time it was the opinion of many surgeons and urologists that penetrating wounds of the lumbar areas with resulting perirenal hemorrhage were best treated conservatively with a compression bandage and that surgical intervention should be reserved only for those cases in which serious renal damage could be demonstrated.40–44

In the early 1950s, although it was hoped that renal biopsy might be beneficial to patient management, this was far from being certain. However, the early use of renal biopsy was spurred by the compelling practical considerations of managing patients with renal failure. The hemodialysis center in Copenhagen, directed by Claus Brun, and one of the few such units in Europe, had a limited number of dialysis machines and a large load of patients. Triage of patients became necessary because only cases of acute and potentially reversible renal failure could be accepted for treatment. It was thought that renal biopsy could provide that information. It was also important to find out whether a renal biopsy could be safely performed in a patient who was soon to be treated with heparin in the course of hemodialysis. It was found that the information provided by the biopsy was most useful in the selection of patients for hemodialysis and that it was safe if the procedure was performed 2 or 3 days before anticoagulation.3

Critique and Opposition

It took several years and many thousands of renal biopsies before this procedure became generally accepted by the medical community. The opposition came from different sources. It was first voiced by urologists, some of whom felt that internists were invading their territory and also resisted that if complications resulted from the procedure, they usually would be called on to handle the problem. However, this opposition was short-lived because it was soon established that needle biopsies were going to be limited to medical diseases of the kidneys, and indeed, renal biopsy, for several different reasons, was contraindicated in surgical diseases such as tumors, cysts, abscesses, nephrolithiasis, and others. Actually, the strongest opposition came from internists and even from some nephrologists. The first criticism was that the risks of the procedure could not be compensated for by the value of the information obtained from the biopsy. By some, this information was thought to be of only academic interest. Further, the question was asked in the early 1950s and is still asked today38,45–48: What is the value of reaching a more accurate diagnosis if there is no effective therapy for most renal diseases? The answer to this question is clear: Medicine would still be in the Middle Ages if an accurate diagnosis first
and then identification of etiologic agents and pathogenetic mechanisms would not have led to the discovery and application of appropriate and effective therapeutic measures for many diseases. Further, in the history of medicine, it is well established that practically all new diagnostic procedures and therapeutic innovations have been associated with some risks to both the patient and the clinician. This is particularly true during the first few years of application of a new procedure. As experience increases and the technique is refined and gradually made less dangerous, the indications for the procedure become more precisely defined and the findings are more accurately interpreted. This is exactly what happened for percutaneous needle biopsy of the kidney.

In retrospect it would also appear that some clinicians were unduly concerned about the risks of needle renal biopsy. In fact, these risks are no greater and probably are less frequent than those of many other generally accepted so-called invasive procedures, such as liver biopsy and catheterization of the urinary bladder among others. This appears to be so when a renal biopsy is taken by a properly trained and experienced physician. It is also true that the value of a renal biopsy is much greater if it is studied by an experienced renal pathologist who is familiar and in control of the three techniques that today are considered necessary in many instances for an accurate interpretation of the specimen: light microscopy, electron microscopy, and immunopatology.

In December 1955, about 3 years after the introduction of percutaneous renal biopsy in the United States, a most critical editorial of this procedure was published in *Lancet.* Robert Kark’s response to the editorial appeared in the same journal in January 1956. It would have been desirable to quote here Kark’s rebuttal in its entirety, but space limitations do not permit it. Some excerpts will have to do. For example, the editor of *Lancet* stated that “in patients with the nephrotic syndrome therapeutic trial [of cortisone or corticotrophin] would give the same information [as renal biopsy] with less disturbance to the patient.” Kark answered: “Unfortunately this is not true, without histologic confirmation the response to cortisone or corticotrophin is always unpredictable; this is hardly surprising if we recognize that ‘nephrotic syndrome’ is but a syndrome with many underlying pathological causes.” In closing, the editor also said, “it seems unlikely that renal biopsy will achieve the importance that liver biopsy is now assuming. Many patients have been saved by liver biopsy in the differential diagnosis of prolonged jaundice; but differential diagnosis of renal disease is rarely of such immediate and crucial importance.” At the time, liver biopsy had been in widespread use for about 20 years and renal biopsy for only 2 or 3 years. Kark’s rebuttal was prophetic and clearly demonstrated his clear thinking and understanding of the problem. He wrote: “On the basis of our experience with both types of biopsies we expect that biopsies of the kidney will be of greater potential value to patients with disease involving the kidneys than hepatic biopsy is to those with jaundice and other types of hepatic disorders.” Kark went on to say that “it is probable that fewer needle biopsies of the kidney will be necessary 20 years hence, for by that time clinical investigators will have sorted out by renal biopsy the early and treatable stages of many diseases which produce the end-stage kidney.” What is remarkable is that Kark’s answer to the editorial in *Lancet* was written in January 1956 when the experience with renal biopsy was still relatively limited and electron microscopy and immunopathology had not made as yet their full contributions.

It is interesting to note that those papers that criticized the use of percutaneous renal biopsy do not seem to have a pathologist among the authors. One also receives the impression that the same authors, although familiar with the diagnostic terminology used in pathology reports of renal biopsies, would have been less critical of the procedure if they had had a more complete working knowledge of the different lesions and other histopathologic features on which a diagnosis is based. The same applies to the terminology often used to qualify lesions such as active and inactive, reversible and irreversible, progressive and nonprogressive, as well as to the relationship of different lesions to functional disturbances. In other
words, one gets the impression that these clinicians would be more supportive of the role of renal biopsy if they would sit at the microscope and exchange views and information with their pathologists. Clinico-pathologic correlations are better established together. It should be recognized that a renal biopsy diagnosis and related, clinically useful morphologic information is best arrived at by a close collaboration between clinician and pathologist. Lack of communication between the two may lead to serious misunderstandings.50

During the first half of the 20th century, physiologists the world over, in the United States best represented by Homer Smith, had made enormous advances in the study and understanding of renal functional mechanisms and had developed methods to measure with great accuracy renal blood flow, glomerular filtration rate, and tubular reabsorption.51 This functional approach to the study of the kidney in health and disease played a major role in the training of clinical nephrologists and greatly influenced their thinking. Indeed, when renal biopsy was first introduced, the thought was expressed by some of them that measurement of renal function by the already available methods was so accurate that the study of renal tissue at the same time was superfluous and not likely to contribute much to the diagnosis and management of the patient with renal disease.36,45 This conflict between the importance of function versus that of morphology (structure) is well discussed by Addis and Oliver11 in the introduction to their book The Renal Lesions in Bright's Disease in which they state that the attitude of many clinicians of the time was “it is more important to know what the kidney does than what it looks like.” Obviously what some clinicians failed to appreciate was that, although discrete renal function tests provided the most accurate data, this was a quantitative and not a qualitative type of information. Simpler tests such as complete urine analysis, including microscopy of the sediment and both qualitative and quantitative study of urine proteins, were more likely to provide more revealing information as to the type of renal disease that had caused abnormal function in the first place.

Thomas Addis, in modern times, had been the principal supporter of urine analysis as a mirror of individual renal diseases and believed strongly that the morphologic aspects of renal disease needed more emphasis.52 Kark's group at the University of Illinois was very much in agreement with this point of view. In fact, an exhibit correlating the results of urine analysis with the histopathologic findings of renal biopsies was presented at a meeting of the American Medical Association (1956). The title of the exhibit was The Lost Art of Urine Analysis. Unfortunately, even today prompt and complete urine analysis in most hospitals in the United States is not given the attention it deserves.54

The New Renal Pathology (Pathologists and Their Problems)

A Question of Adequacy

During the first several years of use of percutaneous needle renal biopsy, one of the major criticisms of this procedure was that the size of the specimen was too small to be truly representative of what was going on in the kidneys. It was said that, as a result, an accurate diagnosis could not be made and that the severity of the lesions would be over- or underestimated. The problem of adequacy of the biopsy specimen was studied in 1955 by Muehrcke and colleagues. Needle specimens were taken from 10 different areas of each of several diseased kidneys obtained at autopsy. For each kidney the histopathologic findings of each needle specimen were compared with those found in the other specimens and in the large tissue wedges taken from the same areas that had been biopsied. The results of this comparative study showed that as long as renal cortex with at least five glomeruli was present in the needle specimens, there was an excellent correlation both as to type and severity of the lesions.54 A more extensive evaluation of the adequacy of needle specimens of the kidney, conducted by a similar method, confirmed these results.55 As might have been expected, this was true for diffuse renal dis-
cases but not for those with a focal distribution of lesions, such as focal segmental glomerulosclerosis and acute or chronic pyelonephritis.

The problem of adequacy of needle renal biopsy specimens appeared to have been settled, but it became an issue again a few years later when the already small biopsy specimen had to be subdivided in order to conduct electron microscopic and immunopathologic studies for which the tissue had to be processed by methods different from those used for light microscopy. The situation at that time is illustrated by the following episode. In 1957 our group had the opportunity to study by sequential renal biopsies the case of a young man with the nephrotic syndrome. In the first biopsy by light microscopy no significant changes were seen. By electron microscopy seven glomeruli were available for study, and all showed complete effacement of the foot processes. A diagnosis of minimal change disease (lipoid nephrosis) was made. The patient was treated with corticosteroids, which resulted in complete remission of the proteinuria. A second biopsy at this time showed essentially normal renal tissue by light microscopy. By electron microscopy the five glomeruli available for study all disclosed restoration of discrete foot processes and normal ultrastructural morphology. This was one of the first ultrastructural demonstrations of reversibility of glomerular lesions, and a paper based on this case was sent to a medical journal with the hope of acceptance and early publication. To our great dismay, the paper was rejected because it was thought that the number of glomeruli studied by electron microscopy was inadequate for definite conclusions. Subsequently, the paper was published in another critically reviewed journal. Today, knowing considerably more about renal diseases than we did 40 years ago, an experienced reviewer would not object even if in a biopsy only two or three glomeruli are studied by electron microscopy, at least in cases of diffuse glomerular disease. In any case, one does not often find more than three or four glomeruli in the minute biopsy fragments processed for electron microscopy.

Today all renal pathologists and clinical nephrologists are fully aware of the limitations imposed by the small size of the tissue specimen available for study. Several steps can be taken to reduce these limitations to a minimum. We have learned the importance of examining serial or semiserial sections from the paraffin block and to look at the same glomeruli at different levels. Every glomerulus in the 1-μm-thick survey sections from the plastic-imbedded tissue for electron microscopy and, if necessary, glomeruli present in the frozen sections from fragments of the biopsy saved for immunopathology are studied in order to increase the adequacy of the specimen. In "desperate" cases we can do electron microscopy in deparaffinized sections and immunopathology in formalin-fixed tissue.

The Living Tissue

The availability of renal tissue obtained from a patient during life when the clinician would like to have information not only on diagnosis, but also on prognosis and on possible response to treatment, compelled the pathologist to evaluate the histopathologic features of a biopsy in a manner quite different from that used for autopsy specimens. Today a clinical nephrologist can expect to be informed if the lesions seen in the biopsy are acute or chronic, active or inactive, reversible or irreversible, and are likely or not to respond to treatment. The nephrologist is also entitled to know about the severity of individual lesions that may differ in their prognostic significance.

In experienced hands, significant complications of percutaneous renal biopsy are extremely rare. Many papers have been written on this topic. There is little that the pathologist can do to help with this problem. Early in my renal biopsy experience I learned to inform the clinician promptly by phone if the specimen was inadequate for diagnosis, if a blood vessel the size of an arcuate artery or larger was included in the specimen, or if tissue other than kidney had been received. Fibrofatty tissue and skeletal muscle were by far the most common nonrenal tissues received but were not considered worrisome. Liver was not infrequently received. Other tissues such as pancreas and adrenals were extremely rare. Interestingly enough, none of these cases, in my ex-
perience, was ever followed by significant complications. The single "lung" case I ever had was that of an eight-year-old child. The specimen I received was that of "compressed" and distorted tissue that at first I recognized only as nonrenal, of which I promptly notified the clinician. Two days later as I studied the slides again to write a brief negative report, the possibility occurred to me that the tissue might be lung. A stain for elastic fibers clearly disclosed a pattern of compressed alveoli. I called the clinician on the case. Absolutely nothing unusual had occurred, and the patient had been discharged feeling perfectly well. Before hemodialysis became readily available, an occasional patient died in renal failure within a few days or weeks after a renal biopsy had been taken. At autopsy evidence of a small subcapsular hematoma near the lower pole of the kidney was frequently found. However, the needle track within the renal parenchyma could not always be located.

The prerequisite of any medical procedure, including renal biopsy, is to be of help to the patient and not only to satisfy the academic curiosity of the physician. This and the desire to obtain the maximal amount of useful information led to the early development and widespread use of analytic methods of evaluation of the biopsy specimen and to a semiquantitative assessment of the severity of individual lesions.\(^{57,58}\) It was also hoped that this approach would help in understanding better the properties of individual cellular elements and other renal structures as well as their role in the histogenesis of different lesions. With the introduction and systematic use of immunopathologic, electron microscopic, and other techniques, this method of studying renal biopsies became more and more challenging.

The semiquantitative analytic method of evaluation of histopathologic lesions would seem to be particularly appropriate to compare serial observations and to establish clinicopathologic correlations. Years before, I had found this method quite useful in sequential studies of wound healing.\(^ {59}\) Results obtained in this manner can be represented in easily interpretable tables and diagrams (histograms).\(^{90–92}\)

Another and perhaps more compelling reason for the introduction of the analytic method of evaluation of renal biopsies was that in the early 1950s my knowledge of renal pathology, like that of other pathologists, was primitive. I needed a system of study that could help me to be comprehensive and place my observations in some order.

In the analytic method, individual structural components of glomeruli, tubules, vessels, and interstitium are studied and the severity of their lesions assessed semiquantitatively on a scale of 0 to 3+ or 0 to 4+. In this manner, the relative contribution of each component to separate diagnostic histopathologic patterns could be established and the nature of the disease clarified. For example, although in the past glomeruli were said to be hypercellular in some diseases, no real effort was made to determine the type of cells and their relative contribution to glomerular hypercellularity. Also the percentage of glomeruli involved by the lesion(s) of a disease process and the localization of lesions within them were determined.\(^{60–62}\)

We and others were fully aware that semiquantitative methods could not possibly be 100 percent accurate and might suffer from individual bias. However, we were able to determine that data obtained in this manner were reproducible even after an interval of several years between observations, at least when the studies were conducted by the same or similarly trained investigators.\(^ {63}\) Also there seemed to be reasonably good agreement, at least for some histopathologic renal features, between quantitative and semiquantitative measurements.\(^ {64}\) Truly quantitative methods (e.g., actual number of cells, diameter of glomeruli, thickness of basement membranes) in standard light microscopic preparations were not done because the unavoidable variations in the size of the biopsy, thickness of sections, difference in the type and quality of fixation, and staining could not possibly yield reliable results.\(^ {65}\) By contrast, tissue preparations for ultrastructural studies are technically much more consistent in quality, and truly quantitative measurements are likely to be considerably more reliable, at least for some renal structures such as the thickness of the glomerular and tubular basement membranes.\(^ {66,67}\)

The analytic method of evaluating renal biopsy
findings has now been in widespread use for many years and has also led to the development of a number of histopathologic indices (e.g., index of severity, index of activity, index of chronicity) that are used as prognostic indicators and to evaluate the effects of therapy. As stated elsewhere, no matter what its shortcomings, this type of evaluation compels the pathologist to be more complete and precise in the study of renal biopsies, resulting in more accurate and informative pathology reports.

During this early period, with the availability of sequential biopsies, the reversibility of many different renal lesions, spontaneously or as a result of treatment, was documented for the first time. I have already mentioned the complete restoration to normal of the principal lesion of minimal change disease after corticosteroid treatment. Also studied at that time was the complete spontaneous resolution of the glomerular and tubular changes of acute poststreptococcal glomerulonephritis, toxemia of pregnancy, and acute tubular necrosis. In this regard, it is relevant to note that today ethical concerns no longer permit performing repeat biopsies, particularly in patients in whom the renal disease has shown much improvement. This is understandable, but it is also one of the reasons why the mechanisms by which renal lesions, spontaneously or after treatment, resolve are still poorly understood.

The New Terminology

Robert Heptinstall, in his review on the development of renal pathology, wrote, "By the middle of the 20th century a great number of anatomical details had been accumulated and crucial experiments performed. But in spite of this there was incredible confusion." There is no question that to a large extent this state of affairs was due to the very imprecise terminology then available and the loose way in which it was applied. Names and classifications of renal diseases were often a combination of clinical, etiologic, and morphologic terms. For example, the terms lipoid nephrosis and nephrotic syndrome were used interchangeably. Morphologically, the words sclerosis, hyalinosis, and fibrosis were often used interchangeably (e.g., sclerotic, hyalinized, and fibrotic glomeruli) because we did not realize that, although these lesions are similar histologically, in fact they are entirely different processes. Subacute glomerulonephritis, a disease characterized by the presence of glomerular cellular crescents, was thought to be a later phase rather than a particularly severe and rapidly progressive form of acute glomerulonephritis. By clarifying for the first time the natural course of many renal diseases, renal biopsy greatly helped to introduce order where previously there was chaos. Electron microscopy and immunopathology, by increasing our knowledge of the fine structure and composition of both normal and abnormal renal components as well as the composition of immune- and nonimmune-related deposits in renal tissue, permitted for the first time the formulation of classifications of renal diseases that could be exclusively morphologic, immunologic, or etiologic and were quite distinct from the clinical classifications. To define the distribution of glomerular lesions the terms focal and diffuse for all glomeruli and segmental (local) and global for individual glomeruli were introduced. The words nephritis and nephropathy were defined to separate an inflammatory from a noninflammatory disease. The term lupus nephritis was coined to single out the special features and complex nature of this type of glomerulonephritis. The term diabetic nephropathy was introduced because it more accurately represented the sum total of all the renal lesions attributable to diabetes, including the intercapillary nodular sclerosis of Kimmelstiel and Wilson. The need to impose order on the then prevailing and most confusing terminology led to the appointment of several committees of "experts" on nomenclature, which under the direction of E. Lowell Becker, Robert Kark, and Kurt Lange eventually produced a book on renal nomenclature. John Merrill was at first quite skeptical about such a need but concluded his perceptive and humorous review by praising the book. Several years later, a comparable updated book was published under the auspices of the World Health Organization.
Technical Advances

Light Microscopy

Shortly after the first needle renal biopsies were received, it was realized that these small tissue specimens required special attention. The time schedule for fixation and clearing used for most specimens in surgical pathology was shortened, and then better fixatives were introduced. Zenker-Formol and particularly alcoholic Bouin became popular. As a result, renal structures were better preserved, and much better results were obtained with several special stains. In addition to hematoxylin–eosin, the trichrome stain of Masson and the periodic acid–Schiff reaction of McManus were used at first. Then Jones introduced the methenamine–silver stain, which gave superior results for basement membranes. Hale’s colloidal iron stain for the mucous substances of the glomerulus was introduced somewhat later. Most importantly, Jones and then Churg and Grishman emphasized the importance of thinner sections (2 to 3 μm), particularly for the visual resolution of glomerular lesions. All these methods became standard procedure over a period of several years. However, recently, many pathologists have gone back to formalin fixation because of convenience; it is less acidic and cross-links tissue antigens less, allowing better preservation of antigenicity for modern immunopathologic and molecular approaches.

Electron Microscopy

Percutaneous needle biopsy had been practiced in many medical centers for several years when two new major technical advances, electron and fluorescent microscopy, began to be applied systematically to the study of renal tissue. This development greatly increased both the scientific and clinical value of renal biopsy. Application of electron microscopy to biologic material had been delayed by the lack of appropriate embedding media for animal tissue. At first, using osmium tetraoxide both as a fixative and as a staining solution, the minute tissue fragments were embedded in methylmethacrylate. Ultrathin sections were cut with glass knives and a special microtome. Later, glutaraldehyde fixation was followed by postfixation in osmium tetroxide, embedding in Epon or other plastic material. Diamond knives were introduced for sectioning. The electron microscopes invented in Germany and first built there and in the United States gradually have been replaced by greatly improved instruments made in Holland or Japan.

The high magnification of transmission electron microscopy applied to the delicately fixed, living tissue at last clarified the complex normal structure of the kidney. It took several years of work by many investigators to achieve this goal. The need for ultrastructural studies to fully understand the complexities of renal structural changes in disease was brought forcefully to my attention when in 1956 in a renal biopsy report of a patient with the idiopathic nephrotic syndrome most unwisely I made a diagnosis of “essentially normal renal tissue” because no significant abnormalities had been seen by light microscopy. I was rather bluntly told by the clinician on the case that my diagnosis could not possibly be correct because that patient was losing protein in the urine at a rate of about 10 g/day. As clearly shown by several studies, it became quite obvious that the electron microscope was needed to resolve the mystery of some renal diseases and related functional disturbances.

In the beginning, in part because the electron microscopic techniques were still somewhat unsatisfactory, it was difficult to distinguish one structure from another and to become oriented in the black and white images seen on the screen of the electron microscope. Techniques were also criticized. In about 1956 at a meeting near Chicago, Vincent Hall, who had been demonstrating electron micrographs of endothelial fenestrae in glomerular capillaries, was told by a prominent professor of pathology during the discussion period that the fenestrae were almost certainly an artifact due to air bubbles in the embedding medium. For a while, mesangial cells, so clearly demonstrated by Zimmerman by light microscopy many years before, could not be found and properly located by the electron microscopists.
quhar and Palade referred to them as "deep endothelial cells," and I also thought for a while that these cells could often be shown to be in direct contact with the capillary lumen.

In those early days and less frequently even today, many structures are seen in electron micrographs of renal tissue whose significance cannot be understood. As might be expected, the observer readily sees and recognizes things that have been seen and described before by others whereas those that are not so unnoticed. In 1972, Gyorkey and colleagues reported the presence of peculiar tubuloreticular structures in the cytoplasm of endothelial cells of glomerular capillaries in renal biopsies from patients with lupus nephritis. At that time I had probably studied by electron microscopy hundreds of renal biopsies from patients with lupus nephritis. However, I could not remember ever having seen such structures. Review of my large collection of electron micrographs from patients with systemic lupus erythematosus promptly confirmed that I was wrong. The tubuloreticular structures, today also known as interferon footprints, were there in good numbers staring in my face in almost every case. Electron microscopy also taught us another thing: many features first recognized by electron microscopy could also be seen by light microscopy if one had good enough histologic preparations and had the patience to study them under oil immersion. A case in point is the well-known spikes of the glomerular basement membrane first seen by electron microscopy in patients with membranous glomerulonephropathy and later shown to be also recognizable by light microscopy in methamine–silver-stained sections.

Gradual improvement in techniques was also an important factor in recognizing a number of lesions that at first could not be identified or were thought to be artifacts. For example, the presence of discontinuities (gaps) of the glomerular basement membrane in several glomerular diseases was first suggested in 1960. This important finding was not accepted by most investigators, but it was amply confirmed many years later by better techniques. However, the precise role of these gaps in hematuria, leukocyturia, and proteinuria still remains to be clarified. The contributions of transmission electron microscopy to the understanding of renal diseases are many and very important.

### Immunopathology

During the first half of the 20th century, it had already been firmly established that immunologic factors played a very important role in the pathogenesis of glomerulonephritis. Masugi had clearly shown that rat kidney antiserum produced in rabbits induced acute proliferative glomerulonephritis when injected into rats. Krakower and Greenspan, of the Department of Pathology at the University of Illinois in Chicago, in rigorously conducted experiments, had demonstrated that the "nephrotoxic" antigen responsible for Masugi nephritis was localized in the glomerular capillary basement membrane. However, there was not proof that a comparable mechanism might be operating in humans. The fluorescent antibody labeling technique of Coons and Kaplan reported in 1950 permitted for the first time identification of antigens and antibodies in freshly harvested animal and human tissue. Within a few years this technique was applied to renal biopsy specimens. As a result, the nature of antibodies, and sometimes even of antigens, could be identified and localized in renal tissue. This permitted characterization of several immunologic and nonimmunologic-mediated renal diseases. During the same period, several immune-mediated experimental models of renal diseases similar to the human entities were created and intensively studied.

### Chicago, Illinois, and the Other Major Centers of Early Renal Biopsy Studies

In the United States percutaneous needle biopsy for the systematic study of renal diseases was first used in two cities. In Washington, DC at the Veterans Administration Hospital, Alvin Parrish,
John Howe, and N. Kramer\textsuperscript{26,116} carried out several valuable investigations among which, of particular interest, were two on clinicopathologic correlations. At the Georgetown University School of Medicine, George Schreiner and colleagues\textsuperscript{116,117} established a very active center for the study of renal diseases. In Chicago in 1952, Kark and Muehrcke, with their new technique of percutaneous needle biopsy, initiated an ambitious program of renal disease studies. They were joined 2 years later by Victor Pollak, a brilliant young internist from South Africa. With the assistance of many clinical research fellows, they conducted a number of studies based on large series of renal biopsies. Notable among them were studies on the nephrotic syndrome, lupus nephritis, diabetic nephropathy, toxemia of pregnancy, and renal vein thrombosis.\textsuperscript{50,61,70,118,119} Emphasis was on clinicopathologic correlations and, having introduced sequential renal biopsies, on the natural history of renal diseases.\textsuperscript{120} Among the pathology residents at the time, Drs. Jose Manaligod and Seymour Rosen took a keen interest in the study of renal biopsies, an interest that they have maintained through their subsequent academic careers. Kark, with his clear vision and grasp of difficult problems, was the leader of the group. Muehrcke and Pollak, with ideas, enthusiasm, and hard work, provided much of the impetus. We all learned a great deal from each other.

The importance of Chicago as a major center of clinicopathologic studies of renal diseases was greatly augmented by the renal biopsy research activity in other local institutions. At Northwestern University, Robert Jennings and David Earle\textsuperscript{71} made important studies of acute post-streptococcal glomerulonephritis and on membranous glomerulonephropathy. At the University of Chicago, Benjamin Spargo,\textsuperscript{121,122} in collaboration with internists and obstetricians, made original observations on toxemia of pregnancy and hypopotassemia.

During the 1951–1961 period, in addition to the Chicago and Washington groups, the most active renal biopsy research centers in the world included

Copenhagen, Denmark: Poul Iversen, Claus Brun, Mogens Bjorneboe, Fleming Raschou, O. Z. Dalgaard, Age Thomsen, and Age Ghormsen

Minneapolis, Minnesota: Robert Vernier, Robert Good, and Marilyn Farquhar

Paris, France: Jean Hamburger, Renee Habib, and Paul Michielsen


Stockholm, Sweden: Hage Bucht and Anders Bergstrand

Pisa and Padova, Italy: E. Fiaschi, G. Ercoli, A. Torsoli, P. Leonardi, and A. Ruol

The CIBA Foundation Symposium on Renal Biopsy

The CIBA Foundation Symposium on Renal Biopsy was held in London in the spring of 1961. At this symposium, 29 clinicians and pathologists critically assessed the risks, values, and potential of percutaneous renal biopsy as an aid to more accurate clinical diagnosis and a guide to therapy.\textsuperscript{7} This meeting, attended by physicians with much experience in the study of renal diseases, was a most significant event and a turning point in the history of renal biopsy. Until then this procedure had been carried out in relatively few centers around the world. Thereafter renal biopsy became an integral part of every major renal center and played a vital role in the birth of nephrology as a major medical specialty.

In addition to Arnold Rich, the chairman of the symposium, the following pathologists attended: A. Bergstrand (Sweden), R. Habib (France), R. H. Heptinstall (Great Britain), R. B. Jennings (United States), H. Z. Movat (Canada), and C. L. Pirani (United States). The combined experience of pathologists and clinicians encompassed about
5,000 biopsies. This was a great improvement on the relatively small series of cases previously reported and appeared to represent a solid base on which to base conclusions. The leisurely exchange of views (over a glass of sherry in midmorning and a nice cup of tea in the afternoon) and frank and detailed discussions of many important issues was extremely useful. For a pathologist, one of the most refreshing aspects of this meeting was to find out how vitally interested and knowledgeable of renal pathology the clinicians were. Indeed, some of the best presentations of biopsy findings were made by clinicians.

Although the diagnostic usefulness of renal biopsy was fully recognized at the CIBA symposium, some doubts were expressed as to its value and benefit to the patient. Even today, more than 30 years after the CIBA symposium and 40 years after the introduction of renal biopsy, I must agree with Arnold Rich that an accurate diagnosis alone will not help the patient unless appropriate therapy is also available. The effectiveness of corticosteroids, immunosuppressants, and other therapeutic agents have now been thoroughly investigated in many renal diseases, but much still remains to be done in this area. Dialysis and transplantation certainly cannot "cure" and should not be considered true "therapy."

Everybody knows that renal biopsy, even as performed today, carries some risks. It is certainly uncomfortable for the patient and expensive. However, the experience of more than 40 years tells us that no matter how complete the clinical and laboratory workup of the patient, even today one cannot predict clinically with precision the type, severity, and activity of the lesions that may be present in the kidneys. It follows that without a renal biopsy no logical choice of therapy can be made or new therapies developed. Further, one could argue that the results of therapy can never be fully evaluated unless repeat biopsies post-treatment are also studied.

Paul Iversen, the father of renal biopsy, was unable to attend the CIBA symposium because of illness. However, during proofreading of the proceedings, he was asked about the reasons for kidney biopsies. His answer is reproduced here in its entirety:

Dr. Brun and I went into the kidney biopsy method because we considered it necessary to learn which patho-anatomical changes were to be found in the kidneys in patients suffering from acute anuria. The changes found in the kidneys of these patients, whose histories are often ambiguous, are sometimes identical in every case, which actually in many cases may lead to the diagnosis, which must presumably always be the object and a condition for treatment. I am also of the opinion that in renal disease you can obtain information through kidney biopsy as to the seriousness of the case, the prognosis, and that of course is always of value for the advice you give your patient. The renal biopsy technique and the judgement of the patho-anatomical changes are so difficult that the procedure and the judgement should only go on at places where there is expert knowledge.

From 1961 to the Present

In the more than 30 years that have elapsed after the CIBA symposium, percutaneous renal biopsy has become more and more a standard procedure in all clinical renal centers. As Robert Kark had predicted, its use has become more selective for the simple reason that now we know a great deal more about renal diseases than we did in the 1950s. An extremely large number of clinicopathologic studies based on renal biopsies have been published, and many problems related to diagnosis, prognosis, etiology, pathogenesis and therapeutic management have been clarified. With the introduction and widespread use of dialysis and transplantation, nephrology has become a full-fledged specialty in internal medicine. In the United States thousands of physicians, having successfully passed the specialty board exami-
nations, have become certified nephrologists. Because of the clinical importance of biopsy findings, sound knowledge of renal pathology has been an important requirement of the Clinical Nephrology Board Examinations. The International Society of Nephrology held its first meeting in 1960 in Evian, France, under the chairmanship of Jean Hamburger. The American Society of Nephrology has grown from about 1,000 members at the time of its first meeting in 1967, chaired by Neal Bricker, to more than 6,000 in 1994. More recently a Renal Pathology Society has been created to provide a forum for discussion of problems of specific interest to pathologists.

As alluded to in previous sections of this chapter, visualization of the kidney and the needle during the biopsy procedure has decreased the risks and increased the percentage of satisfactory biopsies. Light microscopic, electron microscopic, and immunopathologic methods have been refined. New techniques for studying the different cells and structural components of the kidney have been introduced. Among these are scanning and immunoelectron microscopy, use of electron-dense and other tracers, cell culture, microprobe analysis, immunoperoxidase staining, and monoclonal antibodies, and others. Identification of many different receptors and functionally important secretory products of renal cells has increased at a rapid rate. Particularly important in relation to new therapies is the identification of so-called mediators of injury. Molecular biologic methods are now increasingly applied to the study of the “living” tissue provided by the biopsy. Accelerated progress can be expected in our understanding of functional renal mechanisms as well as of the etiology and pathogenesis of individual diseases.

Conclusions

Percutaneous renal biopsy has been an integral part of the diagnostic armamentarium of every nephrology center from the early 1960s and has greatly contributed to the management of the patient with renal disease and even more to the precise identification of different renal diseases and their prognosis. Through immunopathology and electron microscopy, it has also contributed to the clarification of etiology and of pathogenetic mechanisms. Indeed, knowledge and order have been established where once there was ignorance and confusion. At the Sixth Congress of the International Society of Nephrology held in Florence in 1975, Jean Hamburger put it well when with typical French eloquence he said, “The history of nephrology of the last 25 years could be entitled ‘The Decline and Fall of Bright’s Disease and the Birth of Individual Renal Diseases from its Ashes.’” Although renal biopsy was hardly mentioned in his speech, Hamburger in fact was talking about those 25 years when the use of renal biopsy began and became widespread. Intensive, systematic study of these small specimens by light and electron microscopy as well as by immunopathology was the major factor in the demise of Bright’s disease.

Gault and Muehlerko have most properly stated that renal biopsy led “to a quantum change in our knowledge of renal diseases.” This explosion of knowledge was particularly exciting for those of us who had the privilege to work in this field during the early developing years.

It is also relevant to emphasize that a major reason to conduct clinicopathologic studies from the very beginning of renal biopsy was not only to learn more about structural–functional relationships but more importantly to find out whether complete clinical and laboratory workup of the patient could provide accurate information, not only on the severity but also on the type, the etiology, and prognosis of the renal disease. If such a goal could have been achieved, then the need for a renal biopsy for diagnosis and therapy would have been much reduced. Unfortunately, in most instances, this goal has not been achieved and most nephrologists still require a biopsy for the proper management of the patient with renal disease. What is important is that clinical nephrologists and renal pathologists have learned to work closely together for the good of the patient. Structure and function have finally met at the microscope. Whether in the future new, more precise
laboratory tests will provide as accurate information as that provided by a renal biopsy remains to be established. It is hoped that new, effective forms of therapy for individual renal diseases will also be developed in the not too distant future, based on better understanding of the cellular and molecular basis of these conditions.

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